(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
28 August 2003 (28.08.2003)

PCT

(10) International Publication Number
WO 03/070979 A2

(51) International Patent Classification[7]: C12Q 1/68, G01N 33/574

(21) International Application Number: PCT/GB03/00755

(22) International Filing Date: 20 February 2003 (20.02.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

0203998.0	20 February 2002 (20.02.2002)	GB
2002-130927	2 May 2002 (02.05.2002)	JP

(71) Applicant *(for all designated States except US)*: **NCC TECHNOLOGY VENTURES PTE LIMITED** [SG/SG]; 11 Hospital Drive, 169610 Singapore (SG).

(71) Applicant *(for MN only)*: **CRIPPS, Joanna, E.** [GB/GB]; Mewburn Ellis, 1 Redcliff Street, Bristol, Bristol BS1 6NP (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **TAN, Patrick** [SG/SG]; National Cancer Centre, 11 Hospital Drive, 169610 Singapore (SG). **KUN, Yu** [SG/SG]; National Cancer Centre, 11 Hospital Drive, 169610 Singapore (SG). **AGGARWAL, Amit** [SG/SG]; National Cancer Centre, 11 Hosptial Drive, 169610 Singapore (SG). **OOI, Chia, Huey** [SG/SG]; National Cancer Centre, 11 Hospital Drive, 169610 Singapore (SG).

(74) Agents: **CRIPPS, Joanna, E.** et al.; Mewburn Ellis, York House, 23 Kingsway, London, Greater London WC2B 6HP (GB).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/070979 A2

(54) Title: MATERIALS AND METHODS RELATING TO CANCER DIAGNOSIS

(57) Abstract: The invention provides a number of genetic identifiers (genesets) which may be used as diagnostic tools to determine the presence or risk of breast cancer in a patient. The invention also provides genesets which may be used to classify a breast tumour cell as to its molecular subgroup. Each of the identified genesets may be used to product customised specific nucleic acid microarrays for use in diagnosis and classification of breast tumour cells.

MATERIALS AND METHODS RELATING TO CANCER DIAGNOSIS

The present invention concerns materials and methods for diagnosing cancer, especially breast cancer. Particularly, but not exclusively, the invention relates to methods and kits for diagnosing the presence or risk of breast cancer using genetic identifiers.

Carcinoma of the breast is one of the leading causes of death and major illness amongst female populations worldwide. Despite rapid advances in understanding the molecular and genetic events that underlie breast carcinogenesis and the introduction of clinical screening programs, morbidity and mortality due to this disease unfortunately still remains at an unacceptably high level. Indeed, for many parts of the world, breast cancer remains one of the fastest growing cancers in local female populations (Chia et al., 2000). One major challenge in the diagnosis and treatment of breast cancer is its clinical and molecular heterogeneity. Individual breast cancers can exhibit tremendous variations in clinical presentation, disease aggressiveness, and treatment response (Tavassoli and Schitt, 1992), suggesting that this clinical entity may actually represent a conglomerate of many different and distinct cancer subtypes. In addition to variations in clinical behaviour, breast cancer can also display strikingly distinct patterns of incidence in different regional and ethnic populations. For example, in Caucasian populations, the majority of breast cancers occurs in post-menopausal women at a mean and median age of 60 and 61 respectively (Giuliano, 1998). In contrast, studies in

Asian populations show a bi-modal age of incidence pattern beginning at age 40 (Chia et al., 2000, see discussion). Thus, one outstanding question in tumour biology is to explain these regional and ethnic differences on the basis of genetic or environmental factors, and to ascertain if research findings obtained using Caucasian populations can be clinically translated to other ethnic populations as well.

Expression profiling using DNA microarrays has recently proved to be an extremely powerful and versatile approach towards the investigation of multiple aspects of tumour biology. Previous reports using microarrays on breast cancers have focused on the identification of novel tumour subtypes, or on the identification of genes that are differentially expressed between known cancer subgroups (Perou et al., 2000, Gruvberger et al., 2001, Hedenfalk et al., 2001). However, because these studies have primarily focused on samples obtained primarily from Caucasian populations, it is thus an open question if the findings described in these reports will also apply to breast cancers from other ethnic populations. There are also many other key issues also need to be addressed before the use of molecular profiling can become a clinical reality. For instance, there are at present almost no published reports where the expression signatures and molecular subtypes defined in one institution's study have been independently confirmed in a separate series from another centre. Such validations are obviously essential, however, as different health-care institutions are likely to differ in multiple ways which may affect the expression profile of the tumor being studied, such as in the surgical handling of tumor samples, choice of array technology platform, and patient population base. In addition, because it is usually unfeasible to sample the same tumor over an extended period of time, it is often unclear if the different subtypes defined using these approaches truly represent distinct biological entities, or if they represent a single tumor class in different stages of clinical evolution. As one example, there are currently conflicting opinions and data in the field on whether estrogen receptor negative (ER -) breast cancers represent biological entities that have directly arisen from an ER - progenitor cell type in the breast epithelia, or if they have 'evolved' from an originally ER+ state (Kuukasjarri et al., 1996; Parl 2000; Gruvberger et al, 2001).

To address these issues, the inventors have embarked upon a large-scale expression profiling project of breast tumours derived from Asian patients. First, using a combination of supervised and unsupervised clustering methods, they have been able to define a small set of genes which when used in combination serves as a 'genetic identifier' to distinguish if an unknown breast sample is either normal or malignant in a patient of ethnic Chinese descent. The use of such 'genetic identifiers' is of considerable use in the development of molecular diagnostic assays for specific patient populations. Second, using principal component analysis (PCA), the inventors show that the expression profiles of normal breast tissues are considerably less varied than tumour profiles. This finding supports current models of breast tumourigenesis, in which to a first approximation normal breast tissues can be thought of as a relatively constant 'ground state', and that the widely varying expression profiles associated with individual tumours are probably indicative of their arising from this 'ground state' through many different and highly distinct tumourigenic pathways.

Third, by comparing the expression profiles of a series of invasive breast cancers from Chinese patients to published reports using patient samples of primarily Caucasian origin, they found that despite several inter-study methodological differences including choice of array technology platform, many of the key gene signatures and molecular subtypes were remarkably conserved between the two patient populations, suggesting that the molecular subtypes defined using expression-based genomics are indeed highly robust. To the inventors' knowledge, this is the first cross-institution validation study of this type reported for breast cancer.

Fourth, by studying the expression profiles of a series of ductal in-situ cancers (ductal carcinoma in situ, or DCIS), they also found that DCIS tumors express many of the 'hallmark' subtype-specific expression signatures associated with their invasive counterparts. Since DCIS cancers currently represent the earliest non-invasive malignant lesion detectable by conventional histopathology, these results suggest that the molecular subtypes defined in these studies probably arise at a relatively early stage of tumorigenesis (ie pre-invasive) and represent distinct biological entities, rather than a single cancer class in different stages of evolution.

Besides providing a molecular framework for the temporal progression of breast cancer, the inventors' results also support the feasibility of using expression-based genomic technologies for clinical cancer diagnosis and classification across different health-care institutions.

Thus, at its most general, the present invention provides a new diagnostic assay for determining the presence or risk of cancer, particularly breast cancer, in a patient using specific genetic identifiers. Further, the inventors have determined a series of multi-gene classifiers for breast cancer.

In the first instance, the inventors have determined a set of 20 genes (a "genetic identifier") which may be used in combination to predict if an unknown breast tissue sample is either normal or malignant.

In addition to this first geneset (which can distinguish between tumor and normal breast samples), the inventors have also determined other genesets which, can be used as genetic identifiers to classify tumour samples as to subtype. This is of great importance, not only from a research standpoint, but also to ensure the most appropriate treatment is provided.

Thus, the inventors have determined the following genesets which may be used to predict the presence of breast tumour and/or the class of tumour.

1) The geneset provided in Table 2, which when used as a combination, allows a user to predict if an unknown

breast tissue sample is either normal or malignant, particularly using spotted cDNA microarrays.

2) A further set of genes (Table 4a and 4b) which when used in combination can also be used to distinguish between normal and tumour breast tissue samples. This geneset is more preferably used on expression profiles obtained using a commercially available technology platform such as genechips, e.g. Affymetrix U133A Genechips, but can also be utilized employing the spotted cDNA microarray technology described in 1).

3) A set of genes (Table 5a) which when used in combination can predict the Estrogen Receptor status of a confirmed breast tumour sample. A second set of genes (Table 5b) which when used in combination can predict the ERBB2 status of a confirmed breast tumour sample.

4) A set of genes (Table 6) which when used in combination can be used to predict the "molecular subtype" of a breast tumour sample according to the following 5 categories: Luminal, Basal, ERBB2, Normal-like, and ER-negative subtype II. In this embodiment of the present invention, the inventors have used two different types of classification algorithms, namely, (1) one-vs-all (OVA) support vector machines (SVM); and (2) genetic algorithm (GA/maximum likelihood discriminant (MLHD) analysis. Different sets of genes are optimally used depending upon the type of classification algorithm used. Thus, distinct sets of genes are described below for each part.

5) A set of genes (Table 7) which when used in combination can be used to predict luminal subclass in Asian breast cancer patients. The inventors have determined that breast tumours of the "luminal" variety can be "split" into two distinct subtypes Luminal A and Luminal D which are clinically relevant. The genetic identifier (Table 7) is therefore preferably used after the tumour has been formally recognised as "luminal" in nature. This of course, can be achieved using the multi-class predictor of Table 6. The Luminal D tumours are associated with certain expression signatures that are also found highly aggressive non-Luminal tumours, e.g. ERBB2 and Basal. This supports the clinical importance of knowing the tumour subtype.

The determination of specific genesets (genetic identifiers) allows tissue samples to be classified (e.g. tumour v normal) according to the expression pattern of those genes in the tissue. For example, in the first genetic identifier (tumor vs normal) the inventors have determined 10 genes that are usually up-regulated in tumour cells relative to normal cells and 10 genes that are usually down-regulated in tumour cells relative to normal cells. By studying the expression pattern of these particular genetic identifiers, i.e. the composite levels of expression products of these genes in a test sample, it is possible to classify the sample as malignant or normal. Thus, the expression products are able to provide an expression profile or "fingerprint" that can serve to distinguish between normal and malignant cells.

In a first aspect of the present invention, there is provided a method of creating a nucleic acid expression profile for a breast tumour cell comprising the steps of

(a) isolating expression products from said breast tumour cell and a normal breast cell;

(b) identifying the expression profile of a plurality of genes selected from Table 2; for both the tumour and normal cell;

(c) comparing the expression profile of the tumour cell and the normal cell; and

(d) determining a nucleic acid expression profile characteristic of a breast tumour cell.

For the purposes of diagnosis, it is important to obtain an expression profile that is characteristic of a tumour cell, i.e. distinct from the expression profile of the equivalent normal cell. The method according to the first aspect determines the expression profile of a plurality of genes identified by the inventors to be a "genetic identifier" of breast tumour cells (see Table 2).

The expression profile of the individual genes that comprise the genetic identifier will differ slightly between independent samples. However, the inventors have realised that the expression profile of these particular genes that comprise the genetic identifier when used in combination provide a characteristic pattern of expression (expression profile) in a tumour cell that is recognisably different from that in a normal cell.

By creating a number of expression profiles of the genetic identifier from a number of known tumour or normal samples, it is possible to create a library of profiles for both normal and tumour samples. The greater the number of expression profiles, the easier it is to create a reliable characteristic expression profile standard (i.e. including statistical variation) that can be used as a control in a diagnostic assay. Thus, a standard profile may be one that is devised from a plurality of individual expression profiles and devised within statistical variation to represent either the tumour or normal cell profile.

Thus, the method according to the first aspect of the invention comprises the steps of

(a) isolating expression products from a breast tumour cell; contacting said expression products with a plurality of binding members capable of specifically and independently binding to expression products of a plurality of genes selected from Table 2, so as to create a first expression profile of a tumour cell;

(b) isolating expression products from a normal breast cell; contacting said expression products with the plurality of binding members used in step (a), so as to create a comparable second expression profile of a normal breast cell;

(c) comparing the first and second expression profiles to determine an expression profile characteristic of a breast tumour cell.

The expression products are preferably mRNA, or cDNA made from said mRNA. Alternatively, the expression product could be an expressed polypeptide. Identification of the expression profile is preferably carried out using binding members capable of specifically identifying the expression products of genes identified in Table 2. For example, if the expression products are cDNA then the binding members will be nucleic acid probes capable of specifically hybridising to the cDNA.

Preferably, either the expression product or the binding member will be labelled so that binding of the two components can be detected. The label is preferably chosen so as to be able to detect the relative levels/quantity and/or absolute levels/quantity of the expressed product so as to determine the expression profile based on the up-regulation or down-regulation of the individual genes that comprise the genetic identifiers. In other words, it is preferable that the binding members are capable of not only detecting the presence of an expression product but its relative abundance (i.e. the amount of product available).

The determination of the nucleic acid expression profile may be computerised and may be carried out within certain previously set parameters, to avoid false positives and false negatives.

The computer may then be able to provide an expression profile standard characteristic of a normal breast cell and a malignant breast cell as discussed above. The determined expression profiles may then be used to classify breast tissue samples as normal or malignant as a way of diagnosis.

Thus, in a second aspect of the invention, there is provided an expression profile database comprising a plurality of gene expression profiles of both normal and malignant breast cells where the genes are selected from Table 2; retrievably held on a data carrier. Preferably, the expression profiles making up the database are produced by the method according to the first aspect.

With the knowledge of the particular genetic identifiers, it is possible to devise many methods for determining the expression pattern or profile of the genes in a particular test sample of cells. For example, the expressed nucleic acid (RNA, mRNA) can be isolated from the cells using standard molecular biological techniques. The expressed nucleic acid sequences corresponding to the gene members of the genetic identifiers given in Table 2 can then be amplified using nucleic acid primers specific for the expressed sequences in a PCR. If the isolated expressed nucleic acid is mRNA, this can be converted into cDNA for the PCR reaction using standard methods.

The primers may conveniently introduce a label into the amplified nucleic acid so that it may be identified. Ideally, the label is able to indicate the relative quantity or proportion of nucleic acid sequences present after the amplification event, reflecting the relative quantity or proportion present in the original test sample. For example, if the label is fluorescent or radioactive, the intensity of the signal will indicate the relative quantity/proportion or even the absolute quantity, of the expressed sequences. The relative quantities or proportions of the expression products of each of the genetic identifiers will establish a particular expression profile for the test sample. By comparing this profile with known profiles or standard expression profiles, it is possible to determine whether the test sample was from normal breast tissue or malignant breast tissue.

Alternatively, the expression pattern or profile can be determined using binding members capable of binding to the expression products of the genetic identifiers, e.g. mRNA, corresponding cDNA or expressed polypeptide. By labelling either the expression product or the binding member it is possible to identify the relative quantities or proportions of the expression products and determine the expression profile of the genetic identifiers. In this way the sample can be classified as normal or malignant by comparison of the expression profile with known profiles or standards. The binding members may be complementary nucleic acid sequences or specific antibodies. Microarray assays using such binding members are discussed in more detail below.

In a third aspect of the present invention, there is provided a method for determining the presence or risk of breast cancer in a patient comprising the steps of

(a) obtaining expression products from breast tissue cells obtained from a patient suspected of having or at risk of having breast cancer;

(b) contacting said expression products with one or more binding members capable of detecting the presence of an expression product corresponding to one or more genes identified in Table 2; and

(c) determining the presence or risk of breast cancer in said patient based on the binding profile of the expression products from the breast tissue cells to the one or more binding members.

The patient is preferably a woman of Asian descent, e.g. ethnic Chinese descent.

The step of determining the presence or risk of breast cancer may be carried out by a computer which is able to compare the binding profile of the expression products from the breast tissue cells under test with a database of other previously obtained profiles and/or a previously determined "standard" profile which is characteristic of the presence or risk of the tumour. The computer may be programmed to report the statistical similarity between the profile under test and the standard profiles so that a diagnosis may be made.

As mentioned above, the present inventors have identified several key genes which have a different expression pattern in tumour cells as opposed to normal cells of the breast. Collectively, these genes comprise a 'genetic identifier'. The inventors have shown (see below) that the combinatorial expression pattern of the genes belonging to the "genetic identifier" serves to distinguish between normal and tumour cells. Thus, by detecting the expression pattern of the genetic identifier in a breast tissue sample, it is possible to predict the state of the cell (normal or malignant) and whether that patient has or is at risk of developing breast cancer.

The genes that comprise the genetic identifier are given in Table 2. There are 20 genes shown, 10 of which are commonly highly expressed in tumour cells relative to normal cells and 10 of which commonly have decreased expression in tumour cells relative to normal cells. The differential expression of the genes was determined using tumour biopsies and normal tissue biopsies. By detecting the levels of expression products of these genes in a test sample, it is possible to classify the cells as normal or malignant based on the expression profile produced, i.e. an increase or decrease in their expression, relative to a standard pattern or profile seen in normal cells.

Thus, in a further aspect of the invention, there is provided a method of classifying a sample of breast tissue as normal or malignant, said method comprising the steps of

a) obtaining expression products from the cells of the breast tissue sample;

b) contacting said expression products with a plurality of binding members capable of specifically binding to the expression products of a plurality of genes selected from Table 2; and

c) classifying the sample as normal or malignant based on the binding profile of the expression products from the sample and the binding members.

The sample of breast tissue is preferably from a woman of Asian descent, e.g. ethnic Chinese descent.

As before, the expression product may be a transcribed nucleic acid sequence or the expressed polypeptide. The transcribed nucleic acid sequence may be RNA or mRNA. The expression product may also be cDNA produced from said mRNA.

The binding member may a complementary nucleic acid sequence which is capable of specifically binding to the transcribed nucleic acid under suitable hybridisation conditions. Typically, cDNA or oligonucleotide sequences are used.

Where the expression product is the expressed protein, the binding member is preferably an antibody, or molecule comprising an antibody binding domain, specific for said expressed polypeptide.

The binding member may be labelled for detection purposes using standard procedures known in the art. Alternatively, the expression products may be labelled following isolation from the sample under test. A preferred means of detection is using a fluorescent label which can be detected by a light meter. Alternative means of detection include electrical signalling. For example, the Motorola e-sensor system has two probes, a "capture probe" which is freely floating, and a "signalling probe" which is attached to a solid surface which doubles as an electrode surface. Both probes function as binding members to the expression product. When binding occurs, both probes are brought into close proximity with each other resulting in the creation of an electrical signal which can be detected.

As discussed above, the binding members may be oligonucleotide primers for use in a PCR (e.g. multi-plexed PCR) to specifically amplify the number of expressed products of the genetic identifiers. The products would then be analysed on a gel. However, preferably, the binding member a single nucleic acid probe or antibody fixed to a solid support.. The expression products may then be passed over the solid support, thereby bringing them into contact with the binding member. The solid support may be a glass surface, e.g. a microscope slide; beads (Lynx); or fibre-optics. In the case of beads, each binding member may be fixed to an individual bead and they are then contacted with the expression products in solution.

Various methods exist in the art for determining expression profiles for particular gene sets and these can be applied to the present invention. For example, bead-based approaches (Lynx) or molecular bar-codes (Surromed) are known techniques. In these cases, each binding member is attached to a bead or "bar-code" that is individually readable and free-floating to ease contact with the expression products. The binding of the binding members to the expression products (targets) is achieved in solution, after which the tagged beads or bar-codes are passed through a device (e.g. a flow-cytometer) and read.

A further known method of determining expression profiles is instrumentation developed by Illumina, namely, fibre-optics. In this case, each binding member is attached to a specific "address" at the end of a fibre-optic cable. Binding of the expression product to the binding member may induce a fluorescent change which is readable by a device at the other end of the fibre-optic cable.

The present inventors have successfully used a nucleic acid microarray comprising a plurality of nucleic acid sequences fixed to a solid support. By passing nucleic acid sequences representing expressed genes e.g. cDNA, over the microarray, they were able to create an binding profile characteristic of the expression products from tumour cells and normal cells derived from breast tissue.

The present invention further provides a nucleic acid microarray for classifying a breast tissue sample as malignant or normal comprising a solid support housing a plurality of nucleic acid sequences, said nucleic acid sequences being capable of specifically binding to expression products of one or more genes identified in Table 2. The classification of the sample will lead to the diagnosis of breast cancer in a patient. Preferably the solid support will house nucleic acid sequences being capable of specifically and independently binding to expression products of at least 5 genes, more preferably, at least 10 genes or at least 15 genes identified in Table 2. In a most preferred embodiment, the solid support will house nucleic acid sequences being capable of specifically and independently binding to expression products of all 20 genes identified in Table 2.

Typically, high density nucleic acid sequences, usually cDNA or oligonucleotides, are fixed onto very small, discrete areas or spots of a solid support. The solid support is often a microscopic glass side or a membrane filter, coated with a substrate (or chips). The nucleic acid sequences are delivered (or printed), usually by a robotic system, onto the coated solid support and then immobilized or fixed to the support.

In a preferred embodiment, the expression products derived from the sample are labelled, typically using a fluorescent label, and then contacted with the immobilized nucleic acid sequences. Following hybridization, the fluorescent markers are detected using a detector, such as a high resolution laser scanner. In an alternative method, the expression products could be tagged with a non-fluorescent label, e.g. biotin. After hybridisation, the microarray could then be 'stained' with a fluorescent dye that binds/bonds to the first non-fluorescent label (e.g. fluorescently labelled strepavidin, which binds to biotin).

A binding profile indicating a pattern of gene expression (expression pattern or profile) is obtained by analysing the signal emitted from each discrete spot with digital imaging software. The pattern of gene expression of the experimental sample can then be compared with that of a control (i.e. an expression profile from a normal tissue sample) for differential analysis.

As mentioned above, the control or standard, may be one or more expression profiles previously judged to be characteristic of normal or malignant cells. These one or more expression profiles may be retrievable stored on a data carrier as part of a database. This is discussed above. However, it is also possible to introduce a control into the assay procedure. In other words, the test sample may be "spiked" with one or more "synthetic tumour" or "synthetic normal" expression products which can act as controls to be compared with the expression levels of the genetic identifiers in the test sample.

Most microarrays utilize either one or two fluorophores. For two-colour arrays, the most commonly used fluorophores are Cy3 (green channel excitation) and Cy5 (red channel excitation). The object of the microarray image analysis is to extract hybridization signals from each expression product. For one-color arrays, signals are measured as absolute intensities for a given target (essentially for arrays hybridized to a single sample). For two-colour arrays, signals are measured as ratios of two expression products, (e.g. sample and control (controls are otherwise known as a 'reference')) with different fluorescent labels.

The microarray in accordance with the present invention preferably comprises a plurality of discrete spots, each spot containing one or more oligonucleotides and each spot representing a different binding member for an expression product of a gene selected from Table 2. In a preferred embodiment, the microarray will contain 20 spots for each of the 20 genes provided in Table 2. Each spot will comprise a plurality of identical oligonucleotides each capable of binding to an expression product, e.g. mRNA or cDNA, of the gene of Table 2 it is representing.

In a still further aspect of the present invention, there is provided a kit for classifying a breast tissue sample as normal or malignant, said kit comprising one or more binding members capable of specifically binding to an expression product of one or more genes identified in Table 2, and a detection means.

Preferably, the one or more binding members (antibody binding domains or nucleic acid sequences e.g. oligonucleotides) in the kit are fixed to one or more solid supports e.g. a single support for microarray or fibre-optic assays, or multiple supports such as beads. The detection means is preferably a label (radioactive or dye, e.g. fluorescent) for labelling the expression products of the sample under test. The kit may also comprise means for detecting and analysing the binding profile of the expression products under test.

Alternatively, the binding members may be nucleotide primers capable of binding to the expression products of the genes identified in Table 2 such that they can be amplified in a PCR. The primers may further comprise detection means, i.e. labels that can be used to identify the amplified sequences and their abundance relative to other amplified sequences.

The kit may also comprise one or more standard expression profiles retrievably held on a data carrier for comparison with expression profiles of a test sample. The one or more standard expression profiles may be produced according to the first aspect of the present invention.

The present invention further provides a method of diagnosing the presence or risk of breast cancer in a patient of Asian descent, said method comprising

obtaining a breast tissue sample;

isolating expression products from said sample;

labelling said expression products;

contacting said labelled expression products with a plurality of binding members representing a plurality of genes selected from Table 2;

determining the presence or risk of breast cancer in said patient, based on the binding profile of said labelled expression products and the binding members.

The breast tissue sample may be obtained as excisional breast biopsies or fine-needle aspirates.

Again, the expression products are preferably mRNA or cDNA produced from said mRNA. The binding members are preferably oligonucleotides fixed to one or more solid supports in the form of a microarray or beads (see above). The binding profile is preferably analysed by a detector capable of detecting the label used to label the expression products. The determination of the presence or risk of breast cancer can be made by comparing the binding profile of the sample with that of a control e.g. standard expression profiles.

In all of the aspects described above, it is preferred to use binding members capable of specifically binding (and, in the case of nucleic acid primers, amplifying) expression products of all 20 genetic identifiers. This is because the expression levels of all 20 genes make up the expression profile specific for the cells under test. The classification of the expression profile is more reliable the greater number of gene expression levels tested. Thus, preferably expression levels of more than 5 genes selected from Table 2 are assessed, more preferably, more than 10, even more preferably, more than 15 and most preferably all 20 genes.

The genetic identifier (Table 2) mentioned above is particularly suitable for spotted cDNA microarray technology where the microarray (or other similar technology) has been created specifically for this purpose. However, the present inventors have appreciated that the present invention may be modified so that commercially available genechips may be used, rather than going to the trouble of creating one specifically containing the genes identified in Table 2. With this in mind, the inventors have identified a further genetic identifier (Table 5a or 5b) which, although it may be utilized using microarray technology described above, it may also be used on commercially available genechips, e.g. Affymetrix U133A Genechips.

Thus, the aspects of the invention described above may also be carried out using the geneset of Table 4a or 4b instead of that of Table 2 and in addition these may be used on either on commercially available genechips such as Affymetrix U133A Genechips, or using microarray technology described above.

The present inventors have also identified a further set of genes (Table 5a) which may be used to classify a breast tumour on the basis of the Estrogen Receptor (ER) status. This is clinically important as ER^+ tumours can be treated with hormonal therapies (e.g. tamoxifen) and ER^- tumours are typically more aggressive and refractory to treatment.

Likewise, the present inventors have also identified a further set of genes (Table 5b) which may be used to classify a breast tumour on the basis of the ERBB2+ status. Knowing the $ERBB2^+$ status of a breast tumour is also clinically important as $ERBB2^+$ tumours are typically highly aggressive and carry a poor clinical prognosis. ERBB2+ tumors are also candidates for treatment with Herceptin (an anti-cancer drug).

The genesets provided in Tables 5a and 5b were determined by generating expression profiles for a set of breast tumour samples using Affymetrix U133A Genechips. A series of statistical algorithms were used to identify a set of genes that were differentially expressed in ER^+ vs ER^- samples as well as $ERBB2^+$ vs $ERBB2^-$ samples. Accordingly, the present invention further provides genesets which may be used in methods of classifying breast tumours according to ER and ERBB2 status.

Thus, in a further aspect of the present invention, there is provided a method of classifying a breast tumour according to its ER and/or ERBB2 status comprising.

a) obtaining expression products from the tumour cells;

b) contacting said expression products with a plurality of binding members capable of specifically binding to the expression products of a plurality of genes selected from Table 5; and

c) classifying the tumour cell on the basis of ER and/or ERBB2 status based on the binding profile of the expression products from the sample and the binding members.

As with the first aspect of the present invention, the plurality of binding members are preferably nucleic acid sequences and more preferably nucleic acid sequences fixed to a solid support, for example as a nucleic acid microarray. The nucleic acid sequences may be oligonucleotide probes or cDNA sequences.

The tumour cell may be classified according to its ER and/or ERBB2 status on the basis of the expression of the genes identified in Table 5. Table 5 identifies each gene as either being upregulated (+) or down regulated (-) in an ER^+ or $ERBB2^+$ tumour. With this information, it is possible to determine whether the breast tumour cell under test is ER^- or ER^+ and/or $ERBB2^+$ or $ERBB2^-$.

As with all aspects of the present invention, the plurality of genes selected from the determined genesets (Tables 2-7 with the exception of Table 6b) may vary in actual number. It is preferable to use at least 5 genes, more preferably at least 10 genes in order to carry out the invention. Of course, the known microarray and genechip technologies allow large numbers of binding members to be utilized. Therefore, the more preferred method would be to use binding members representing all of the genes in each geneset. However, the skilled person will appreciate that a proportion of these genes may be omitted and the method still carried out in a reliable and statistically accurate fashion. In most cases, it would be preferable to use binding members representing at least 70%, 80% or 90% of the genes in each respective geneset.

In a further aspect of the invention, there is provided a method of classifying a breast tumour cell as to its molecular subtype comprising

a) obtaining expression products from the tumour cells;

b) contacting said expression products with a plurality of binding members capable of specifically binding to the expression products of a plurality of genes selected from Table 6; and

c) classifying the tumour cell with regard to its molecular subtype based on the binding profile of the expression products from the tumour cell and the binding members.

The molecular subtypes are preferably Luminal, ERBB2, Basal, ER-type II and Normal/normal like. These sub-types are defined in the following text.

In practice, the expression profile of the tumour sample to be classified is determined using the genesets described in Table 6 (Table 6a or 6b depends on the type of classification algorithm used). Secondly, the expression profile would be compared to a database of "references" (control profiles, where each "reference" (control) profiles, where each "reference" profile corresponds to the "average" tumour belonging to that particular molecular type. In this case, rather than just having normal and tumour, or ER^+ and ER^-, the "reference" profiles will correspond to five distinct subtypes. Third, by using a suitable classification algorithm, the unknown tumour sample can be assigned to the specific subtype for which the expression profile finds a good reference match.

Where the plurality of binding members are selected as being capable of binding to the expression products of a plurality of genes from Table 6a, the number of binding members used will govern the reliability of the test. In other words, it is not necessary to use binding members capable of specifically and independently to <u>all</u> genes identified in Table 6a, but the more binding members used, the better the test. Therefore, by plurality it is meant preferably at least 50%, more preferably at least 70% and even more preferably at least 90% of the genes as mentioned above.

In a still further aspect of the invention, there is provided a method of further sub-classifying a breast tumour cell as either luminal A or luminal D subtype comprising

a) obtaining expression products from the tumour cells;

(b) contacting said expression products with a plurality of binding members capable of specifically binding to the expression products of a plurality of genes selected from Table 7; and

c) classifying the tumour cell with regard to its molecular subtype based on the binding profile of the expression products from the tumour cell and the binding members.

Preferably, the method is carried out on expression products obtained from a breast tumour cell which has already been classified as "luminal", e.g. using the genetic identifier of Table 6a or 6b.

With regard to the geneset provided in Table 6b, it is preferable that all of the genes in the geneset are used for classification. The reduction in the number of genes will take away the likelihood of a reliable result. This is because this geneset is selected using the genetic algorithm approach.

The inventors have provided a number of genetic identifiers (Tables 2 to 7) which can be used to diagnose and/or predict risk of breast cancer and, further, can be used to classify the type of breast cancer, particularly for women of Asian descent.

The provision of these genetic identifiers allows diagnostic tools, e.g. nucleic acid microarrays to be custom made and used to predict, diagnose or subtype tumours. Further, such diagnostic tools may be used in conjunction with a computer which is programmed to determine the expression profile obtained using the diagnostic tool (e.g. microarray) and compare it to a "standard" expression profile characteristic of normal v tumour and/or molecular subtypes depending on the particular genetic identifier used. In doing so, the computer not only provides the user with information which may be used diagnose the presence or type of a tumour in a patient, but at the same time, the computer obtains a further expression profile by which to determine the "standard " expression profile and so can update its own database.

Thus, the invention allows, for the first time, specialized chips (microarrays) to be made containing probes corresponding to the genesets identified in Tables 2 to 7. The exact physical structure of the array may vary and range from oligonucleotide probes attached to a 2-dimensional solid substrate to free-floating probes which have been individually "tagged" with a unique label, e.g. "bar code".

A database corresponding to the various biological classifications (e.g. normal, tumour, molecular subtype etc.) may be created which will consist of the expression profiles of various breast tissues as determined by the specialized microarrays. The database may then be processed and analysed such that it will eventually contain (i) the numerical data corresponding to each expression profile in the database, (ii) a "standard" profile which functions as the canonical profile for that particular classification; and (iii) data representing the observed statistical variation of the individual profiles to the "standard" profile.

In practice, to evaluate a patient's sample, the expression products of that patient's breast cells (obtained via excisional biopsy or find needle aspirate) will first be isolated, and the expression profile of that cell determined using the specialized microarray. To classify the patient's sample, the expression profile of the patient's sample will be queried against the database described above. Querying can be done in a direct or indirect manner. The "direct" manner is where the patient's expression profile is directly compared to other individual expression profiles in the database to determined which profile (and hence which classification) delivers the best match. Alternatively, the querying may be done more "indirectly", for example, the patient expression profile could be compared against simply the "standard" profile in the database. The advantage of the indirect approach is that the "standard" profiles, because they represent the aggregate of many individual profiles, will be much less data intensive and may be stored on a relatively inexpensive computer system which may then form part of the kit (i.e. in association with the microarrays) in accordance with the present invention. In the direct approach, it is likely that the data carrier will be of a much larger scale (e.g. a computer server) as many individual profiles will have to be stored.

By comparing the patient expression profile to the standard profile (indirect approach) and the pre-determined statistical variation in the population, it will also be possible to deliver a "confidence value" as to how closely the patient expression profile matches the "standard" canonical profile. This value will provide the clinician with valuable information on the trustworthiness of the classification, and, for example, whether or not the analysis should be repeated.

As mentioned above, it is also possible to store the patient expression profiles on the database, and these may be used at any time to update the database.

Aspects and embodiments of the present invention will now be illustrated, by way of example, with reference to the accompanying figures. Further aspects and embodiments will be apparent to those skilled in the art. All documents mentioned in this text are incorporated herein by reference

Figure 1: Unsupervised Partitioning of Normal and Tumour Breast Samples. Individual expression profiles were subjected to standard data selection filters (see text), and the resultant data matrix, comprising approximately 800 array targets, was sorted using hierarchical clustering. Normal samples ('xxxN') are underlined, while tumour samples ('xxxT') are not. Numbers represent the NCC Tissue

Repository numbers associated with each sample. The dendogram branches illustrate the extent of similarity between the biological samples. Normal and Tumour samples segregate independently, but only at secondary levels of the dendogram. Minor variations on the data filters used to select this data set also yielded highly similar dendograms (P. Tan, unpublished observations)

Figure 2: Improvement of Normal and Tumour Sample Partitioning Using Combined Outlier Genesets (COG). (A) Independent outlier genesets for normal (left) and tumour (right) samples were defined. Each clustergram consists of a matrix of array targets (rows) by biological samples (columns), and light grey represents upregulation, while dark grey represents downregulation (see Materials and Methods for selection criteria). The outlier geneset for normal samples consists of 60 genes, while the outlier geneset for tumour samples consists of 75 genes. Specific normal and tumour samples used in the establishment of the outlier genesets are listed below each clustergram. Underlined sample numbers indicate reciprocal hybridizations, where the tumour/normal sample was labelled using Cy5 and the reference sample Cy3. (B) Partitioning of normal and tumour samples using the COG. The 108 unique array targets comprising the COG were used to segregate the tumour and normal samples from Figure 1 using standard hierarchical clustering. In contrast to Figure 1, division of the normal (xxxN) and tumour (xxxT) samples is now observed as a primary class division, with 2 misclassifications.

Figure 3: Partitioning of Normal and Tumour Samples using a Minimal 20-Element Genetic Identifier. The 20 array targets from the COG (Table 2) that were most highly correlated to the tumour/normal class distinction were used to segregate (A) the training set from Figures 1 and 2b, and (B) a naïve test set of 10 normals and 11 tumours. In both cases, accurate segregation of normal and tumour samples at the level of the primary class division can be observed.

Figure 4: Comparison of expression profile variation in normal and tumour samples. Independent normal and tumour datasets were established using the combined samples of Figure 3a and 3b (total = 48 samples). Using PCA, the entire gene expression matrix of approximately 8000 array targets in these datasets were reduced to basic principal components. The extent of variance of each component normalized to the 1st component (normalized eigenvalue) is depicted on the y-axis, and the principal component number on the x-axis, beginning with the 2nd component (since the first component of each set is 1). To observe the rate of 'decay' of information, the components for each dataset are depicted in decreasing order of variance. Normal samples consistently exhibit a lower information decay rate across their components compared with tumours.

Figure 5: Gene expression patterns of 62 samples including 56 carcinomas and 6 normal tissues, analyzed by hierarchical clustering using different gene sets. Samples were divided into 6 subtypes based on differences in gene expression (legend), and are : Luminal , (S1); ERBB2+/ER+ (S2, ERBB2+/er- (S3), Basal-like (S4), ER negative subtype II (S5), and Normal/Normal-like (S6)

(a) Unsupervised hierarchical clustering using a dataset of 1796 genes. The gray underline indicates a cluster which contains a mixture of Luminal and ERBB2+/ER+ samples. **(b)** Semi-supervised hierarchical clustering using the 'common intrinsic gene set' (CIS, 292 genes). **(c)** The full cluster diagram using the CIS. Shaded bars to the right of the clustergram represent gene clusters A-E (Table 3), and are (A) Luminal epithelial genes with ER. (B) 'Novel' genes. (C) Basal epithelial genes. (D) Normal breast-like genes. (E) ERBB2-related genes.

Figure 6 (a)-(d) Representative Examples of DCIS Samples Used in this Study. Two samples are shown (a)/(b), and (c)/(d). The DCIS status of each sample was confirmed both by examination of paraffin H & E sections of samples ((a) and (c), HE), as well as frozen cryosections ((b) and (d), FS) of the actual sample that was processed for expression profiling. (e) 'Distinct Origins' and 'Evolutionary' Theories of Breast Cancer Development. The 'Distinct Origins' hypothesis proposes that different molecular subtypes of cancer arise via different tumorigenic pathways, and thus constitute distinct biological entities. The 'Evolutionary' hypothesis proposes that the different molecular subtypes arise as a result of a single (or a few) cancer classes undergoing different stages of phenotypic development. One cannot distinguish between the two hypotheses by only studying advanced invasive cancers obtained at a single point in time.

Figure 7: DCIS samples express the hallmark genes of advanced carcinoma subtypes. DCIS samples are shown as dark vertical lines. Based upon the CIS geneset, six out of

twelve DCIS samples cluster within the ERBB2+ groups (S2 and S3), 5 samples in the Luminal group, and one sample was in the normal-like group. Shaded bars to the right of the clustergram represent the same gene clusters as shown in Figure 5. (A) Luminal epithelial genes with ER. (B) Basal epithelial genes. (C) Normal breast-like genes. (D) ERBB2.

Figure 8: Summary of pathway-specific and overlapping genes for the Luminal A and ERBB2+ tumor subtypes. 'U' indicates upregulated genes and 'D' indicates downregulated genes. For example, there are 245 genes upregulated and 705 genes downregulated during the normal/DCIS (Luminal) transition. Numbers in bold are overlapping genes between two gene sets. **a**) Results based upon a false-discovery rate (FDR) of 5%. **b**) Results when only the top 100 most significantly regulated unique genes are compared.

Figure 9. a) Discovery of a Luminal D subtype. A series of previously homogenous Luminal A tumors (identified as subtype S1 by the CIS in Figures 5 and 7 were regrouped by hierarchical clustering based upon 'proliferation cluster' linked genes. Two broad groups are observed, which exhibit low (Luminal A) and high (Luminal D) levels of expression of the 'proliferation cluster' respectively. **b**) High levels of the 36-gene 'proliferation cluster' is also observed in other aggressive tumor types. **Luminal D** (15 out of 17 samples, indicated as dark bars under sample numbers), Basal (ER-) and ERBB2+ve samples all strongly express the 36-gene 'proliferation cluster' (bar below clustergram, left branch), while Luminal A (all but one boundary case), normal-like and normals are show low levels of expression. Light grey/white indicates upregulation, while dark grey/black indicates downregulation.

Materials and Methods

Breast Tissue Samples

Primary breast tissues were obtained from the NCC Tissue Repository, after appropriate approvals had been obtained from the institution's Repository and Ethics Committees. In general, all tumour and matched normal tissues were simultaneously harvested during surgical excision of the tumour. After surgical excision, the samples were immediately grossly dissected in the operating theatre, and flash-frozen in liquid N2. Histological confirmation of tumour status was subsequently provided by the Dept of Pathology at Singapore General Hospital. Samples were stored in liquid N2 until processing was performed. With the exception of 1 tumour and matched normal sample pair that came from an Indian patient, all other samples were derived from Chinese patients. Confirmation of the DCIS status of tissue samples used in this report was achieved both by conventional H & E staining of archival samples, as well as direct cryosections of the actual sample that was processed for expression profiling.

Sample Preparation and Microarray Hybridization

For hybridisations involving Affymetrix Genechips, RNA was extracted from tissues using Trizol reagent, purified through a Qiagen Spin Column, and processed for Affymetrix Genechip hybridization according to the manufacturer's instructions. For each spotted cDNA microarray hybridization 2-3 μg of total RNA was used following single-round linear amplification (Wang et al., 2000). All breast samples for the spotted cDNA microarray hybridisations were compared against a standard commercially available mRNA reference pool (Strategene) that had been similarly amplified. cDNA microarrays were fabricated following standard procedures (DeRisi et al., 1997), using cDNA clones obtained from various commercial vendors (Incyte, Research Genetics). Except where mentioned, samples were fluorescently labelled using Cy3 dye, while the reference was labelled with Cy5. Hybridizations were performed using Affymetrix U133A Genechips. After hybridization, microarray images were captured using a CCD-based microarray scanner (Applied Precision, Inc).

Data Processing and Analysis

For spotted cDNA microarray data, fluoresence intensities corresponding to individual microarrays were uploaded into a centralized Oracle 8i database. Establishment of various data sets and gene retrievals were performed using standard SQL queries. Hierarchical clustering was performed using the program Xcluster (Stanford) and visualized using the program Treeview (Eisen et al., 1998). To identify outlier genes in tumour and normal datasets, array elements were chosen which consistently exhibited greater than 3-fold regulation across 90% of all arrays for the normal dataset and 80% of all arrays for the tumour dataset. Correlation analysis was performed using the similarity metric concept employed in Golub et. al. (1999). Briefly, the similarity metrics corresponding to the normal/tumour class distinction were calculated for each gene, and the genes then sorted based on descending order of their similarity values. After being sorted by their positive and negative correlation to the class distinction, the top 10 genes from each class were chosen for subsequent cluster analysis. Principal Component Analysis (PCA) was performed by linearly transforming the gene expression matrix, which consists of a number of correlated variables, into a 'smaller' number of uncorrelated variables (principal components). For datasets in linear subspace, the data can be 'compressed' in this manner without losing too much information while simplifying the data representation. The first principal component accounts for maximum variability in the data, and each succeeding component accounts for parts of the remaining variability.

For Affymetrix Genechips, Raw Genechip scans were quality controlled using a commercially available software program (Genedata Refiner) and deposited into a central data storage facility. The expression data was filtered by removing genes whose expression was absent in all samples (ie 'A' calls), subjected to a log2 transformation, and normalized by median centering all remaining genes and samples. Data analysis was then performed either using the Genedata Expressionist software analysis package or using conventional spreadsheet applications. The unsupervised dataset of 1796 genes used in Figure 1 was established by selecting genes exhbiting a standard deviation (SD) of >1 across all well-measured samples. Average-linkage hierarchical clustering, was applied by using the CLUSTER program and the results were displayed by using TREEVIEW (9). Significance analysis of microarrays (SAM) was performed essentially as described in Tusher et al., (2001) (10), using a fold-change cutoff of 2 and an appropriate delta value to cap the gene false-discovery rate (FDR) at 5% (0.05).

Creation of a Common Intrinsic Geneset (CIS)

Genes common to both the U133A Genechip Probe Set and the 'intrinsic' dataset as defined in Perou et al., (2000) were selected in the following manner : Out of the original 'intrinsic' set consisting of 456 cDNA clones, 428 could be assigned to a specific Unigene cluster using the Stanford Source database (Unigene Build 156). This number was then reduced to 403 genes after the removal of duplicate genes. The U133A Genechip probe set was then queried using this list, yielding 292 matches, or 72.5% of the original 'intrinsic' set (counting only unique genes).

Results

Partitioning of Normal and Tumour Breast Specimens Using Unsupervised Clustering

The inventors used cDNA microarrays of approximately 13,000 elements to generate gene expression profiles for a set of 26 grossly-dissected breast tissue specimens (14 tumour, 12 normal) obtained from patients of primarily Chinese ethnicity (see Materials and Methods). After hybridization and scanning, approximately 8,000 array elements were found to exhibit flourescence signals significantly above background levels, and these elements were used for subsequent analysis. Initially, the inventors found that an unsupervised clustering methodology based upon a number of commonly used data filters (e.g. selecting genes exhibiting at least 3-fold regulation across at least 4-5 arrays) (see Perou et al., 1999, Wang et al., 2000) resulted in an array clustergram shown in Figure 1. Specifically, the sample set segregated into two broad groups, with each group consisting of a mixture of tumour and normal specimens. However, within each group, the inventors found that the tumour and normal tissues effectively segregated into fairly independent sub-branches. The observation that tumour and normal tissues can be segregated using unsupervised clustering suggests that specific genes may exist that can effectively distinguish between a tumour and normal sample. However, in the context of a large unsupervised data set, it is also clear that these genes are only capable of distinguishing between normal and tumour samples in sub-branches of the correlation dendogram, rather than at the level of a primary class division. Similar findings have also been reported in other breast cancer expression profiling projects (Perou et al., 2000), suggesting that at the level of global transcriptosome, the expression levels of other genes may 'supercede' the information encoded by genes involved in the tumour/normal class distinction (see discussion).

Use of Outlier Genesets to Classify Normal and Tumour Samples

One of the main objectives of the inventors' research is to identify genes or gene subsets that are of significant diagnostic or therapeutic potential. To be of clinical utility, it will be necessary to identify a class of genes that can accurately predict if an unknown breast tissue sample is normal or malignant at the level of the primary, rather than secondary, class division. To identify these genesets, or 'genetic identifiers', a number of supervised learning strategies, such as neigborhood analysis and artificial neural networks, have been previously described (Golub et al., 1999, Khan et al., 2001). However, the inventors used a slightly different strategy to identify these elements that focuses on the use of highly reproducible outlier genes. In this methodology, samples belonging to different classes are initially established as independent datasets. Within each group, genes that are consistently up or downregulated ('outliers') across all or close to all arrays are then identified. These separate 'outlier groups' are then combined, and the ability of the combined set of genes to distinguish between the two classes is then assessed using standard clustering methodologies.

The inventors first established outlier gene subsets for both the normal and tumour populations. To avoid biases that might be introduced by fluorophore labelling, they also included in each group 5 'reciprocal' expression profiles in which the sample and reference RNA population were inversely labelled. This analysis identified 60 highly reproducible 'outlier' genes for the normal group and 75 genes for the tumour group that were either consistently up or down-regulated across all or close to all arrays (Figure 2). A cross-comparison of the normal and tumour outlier sets revealed a number of genes in common between both sets (Table 1), leading to a final combined outlier geneset (referred to as the COG) of 108 genes.

The COG was then used to cluster the 26 breast tissue samples. In contrast to the large-scale clustergram observed in Figure 1, the inventors found that clustering using the genes found in the COG effectively segregated the majority of tumour and normal samples into two principal branches, with 2 mis-classifications (Figure 2a). Specifically, 1 normal sample and 1 tumour sample were mis-assigned, and in the former case a quality check of the gene expression values revealed that this sample was associated with a number of so-called 'missing' values (grey bars in clustergram), which may have led to this sample being mis-classified. Nevertheless, the majority of samples were correctly grouped, suggesting that for certain datasets, 'outlier analysis' may serve as a simple and effective method to identify discriminating genes between distinct classes.

Definition of a Minimal Genetic Identifier for the Normal vs Tumour Class Distinction in Breast Tissues

Despite representing a dramatic reduction in the number of genes from the initial data set (8,000 to 108), the number of elements contained in the COG is still too large to be feasibly included in its entirety as part of a potential diagnostic assay. Ideally, a diagnostic geneset should consist of i) a minimal number of elements, ii) be of high predictive accuracy, and iii) represent a mixture of genes that are positively and negatively correlated to the class distinction in question. To further reduce the combined outlier geneset to its most informative elements, the inventors used correlation analysis to identify and rank genes in the COG that are most highly correlated to the tumour/normal class distinction (see Materials and Methods). The 10 most highly positively and negatively correlated genes were then assessed in their ability to accurately classify the breast samples. The inventors found that this minimal set of 20 genes, referred to as a 'genetic identifier, accurately classified all of the normal and tumour samples (Figure 2b and Table 2). The genes that make up the 'genetic predictor' represent a mixture of genes known to be involved in breast and tumour biology, as well as other genes whose role in tumour formation have not as yet been described (see discussion).

Predictive Capacity of the 20-gene 'Genetic Identifier'

All analyses done up to this point were performed on the same 'training' set of 26 breast samples, and thus the predictive power of the 20-element geneset has not been addressed. To assess the robustness of this 'genetic identifier', the inventors followed the strategy of Golub et al (1999) and tested the ability of the minimal predictor to classify a naïve 'test set' of another 22 breast samples, of which 12 samples were tumours and the remaining 10 were non-malignant. In a similar fashion to the training set, they found that the 20-gene genetic identifier was also able to classify the naïve set with complete accuracy (Figure 3b). Thus, it appears that the ability of the 'genetic identifier to predict if a given breast sample is normal or malignant is not confined to the training-set from which it was generated. Instead, the number of elements in this geneset, although minimal, may be of sufficient sensitivity and informative power to give it predictive value.

Assessing the Global Level of Variation between Normal and Tumour Breast Tissues

Breast tumours are clinically characterized by wide variations in clinical courses, disease aggressiveness, and response to medication. Consistent with these wide phenotypic variations has been the finding that individual breast tumours can exhibit large variations in their global gene expression patterns (Perou et al., 2000). One common hypothesis to explain these wide variations is to consider them as the consequences of multiple independent pathways of tumourigenesis. However, normal breast tissues are also highly environmentally and hormonally sensitive, and the specific state of a normal breast tissue in a particular patient is often dependent upon numerous demographic factors, such as age, menopausal status, and medication history. Thus, it is formally possible that a certain amount of the variations in expression state observed in tumours may also be reflected in non-malignant breast tissue as well. Since the inventors' data set consists of both normal and malignant samples, they were able to compare the inherent variability of normal and tumour samples to each other. To perform this comparison, they utilized principal component analysis (PCA) on the entire 8,000 gene expression matrix, comprising a total of 22 non-malignant and 26 tumour specimens. Using PCA, the inventors reduced the total gene set to a series of distinct 'components', in which each component represents a finite amount of gene expression variation across the primary data set. They hypothesized that observed variation in the data could arise from multiple sources, such as intrinsic biological variation, as well as experimentally introduced variation (such as differences in sample harvesting, hybridization and labelling conditions, etc). However, since the normal and tumour samples were identically harvested, treated and processed in their experiments, variations due to experimental conditions and handling should be equally shared between both groups. Thus, any differences in variation between the tumour and normal groups can most likely be attributed to intrinsic biological variation.

The inventors plotted the amount of variation observed in the normal and tumour data sets against their principal components (Figure 4). In order to effectively compare the two datasets, each component was normalized to the first component in that dataset, resulting in a graph that depicts how the total variation across the dataset 'decays' with each successive principal component (By convention, the first principal component is usually taken to represent the elements that exhibit maximal variation across the dataset). The inventors observed that as a general rule, every component corresponding to the tumour data set consistently exhibited higher variation than an analogous component in the normal data set. This data indicates that the gene expression profiles of normal breast samples are significantly more 'static' or 'unchanging' when compared to tumour profiles, supporting the hypothesis that the wide variations in gene expression observed in tumours may be a consequence of breast tumours arising from multiple tumourgenic pathways.

Conservation of Molecular Subtypes of Breast Cancer Across Distinct Ethnic Populations

The inventors then used Affymetrix Genechips to profile 56 invasive breast cancers and 6 normal breast tissues that had been isolated from Chinese patients. The raw expression profile scans were subjected to one round of quality control, data filtering and processing (see Materials and Methods), and an unsupervised hierarchical clustering algorithm was used to order the normalized profiles to one another on the basis of their transcriptional similarity. Using a dataset of 1796 genes, which constitute genes that are both well-measured across at least 70% of all samples and which exhibited considerable transcriptional variation across the samples (as reflected by having a high standard deviation), the inventors observed that the majority of the samples segregated into several discernible groups that could be correlated to specific histopathological parameters. For example, many of the ER + tumors clustered together ((S1) bar, Figure 5a), as did the ERBB2 +/ ER - samples ((S3) bar). The normal breast samples also clustered as a discernible group whose individual members exhibited very high correlation to one another, suggesting that there is less transcriptional variation in normal breast tissues as compared to tumors. A number of samples, however, were not accurately segregated by the unsupervised clustering algorithm (gray bar) - it is possible that such 'mixed clustering' results may be attributable to 'noise' contributed by non-malignant components in the primary tissue sample, such as normal breast epithelial tissue, lymphocytic infiltrates, and reactive desmoplastic tissue. As previously mentioned, a similar observation was obtained using the cDNA microarray platform, suggesting that this phenomena is technology-platform independent.

One objective of this study was to determine if the molecular subtypes and associated expression signatures defined in previous published studies were also detectable in a separate patient population. The inventors focused on correlating their expression results to that of Perou et al (2000), a landmark study in which a similar analysis had been performed on a series of breast cancer specimens derived from US and Norwegian patients. Briefly, in that study and a subsequent companion report (Sorlie et al., 2001), the authors determined that invasive breast cancers could be subdivided into at least 5 distinct molecular subtypes based upon an 'intrinsic' geneset representing genes whose transcriptional variation is primarily due to the malignant tumor component. The specific expression signatures that represent the 'hallmark' elements of each particular subtype are summarized in Table 1 (this dataset is henceafter referred to as the Stanford study). Between the Stanford study and the inventors work, there are several differences in methodology and experimental design, such as differences in sample handling protocols, patient population, and expression array platform (2-color cDNA microarray in the Stanford study vs 1-color Genechips in the inventors' study, as well as different array probe sequences). The availability of two distinct breast cancer expression datasets from independent institutions (Stanford and the inventors) thus allowed the inventors to test whether, despite these differences, if the molecular subtypes defined in one institution's experiments are indeed sufficiently robust to be detectable in another institution's study.

To perform this analysis, the inventors first identified probes on the Affymetrix U133A Genechip corresponding to genes belonging to the 'intrinsic' set as defined by the Stanford study (see Materials and Methods). Of 403 unique genes found in the Stanford 'intrinsic' set, 292 genes, or 72.5% of the intrinsic set, were also found on the Genechip array. The inventors henceforth refer to this overlapping set of genes as the 'common intrinsic set' (CIS). Importantly, the CIS still contains many of the 'hallmark' genes whose transcription was reported in the Stanford study to be useful for discriminating between subtype, and reclustering of the Stanford tumors using the CIS also yielded highly similar groupings to that obtained using the full intrinsic set (data not shown). When the invasive cancers in the inventors' series were reclustered on the basis of the CIS, they observed a striking improvement in the segregation pattern where now all the cancer samples grouped into highly distinct classes. The inventors then proceeded to compare the molecular subtypes defined in their study to those discovered by the Stanford study (Luminal A, Luminal B/C, Basal, Normal-like, and ERBB2+) (Perou et al., 2000; Sorlie et al., 2001).

Luminal subtypes : All of the cancers in this group were ER + by conventional immunohistochemisty. The Stanford study defined at least two groups of luminal tumors - Luminal A and Luminal B/C, the latter being associated with a poorer clinical prognosis (Luminal B and C tumors are treated as a single class, as it is reportedly difficult to divide them into two discrete groups (Sorlie et al., 2001). Consistent with the Stanford study, the inventors also observed the presence of a robust Luminal molecular subtype that was highly similar to the Luminal A subtype of the Standford study, as this subtype was characterized by high levels of expression of ER and related genes such as GATA3, HNF3a, and X-box Binding Protein 1 (bar (S1). They could not, however, clearly determine if the Luminal B/C subtypes as defined by the Standford study were also present in their patient population, based upon the criteria that both the B/C subtypes are associated with intermediate levels of ER related gene expression, and that the luminal C subtype also expresses high levels of a 'novel' gene cluster. The inventors also observed the presence of a second luminal subclass (ER+ /ERBB2+) which was distinct from the luminal A cancers in that this other subclass expressed intermediate levels of ER-related genes (similar to Luminal B/C) and genes found in the 'novel' cluster (similar to luminal C, bar (S2). This subclass, however, also expressed high levels of ERBB2-related genes, and is thus likely to be distinct from the luminal C cancers defined by the Stanford study, as luminal C cancers express low levels of the ERBB2 gene cluster. Taken collectively, the inventors' results indicate that Luminal A tumors ("Luminal in Fig. 5) constitute a robust molecular subtype that can be commonly found across different patient populations. Conversely, the luminal B/C and ER+/ ERBB2 +ve subtypes may represent less robust variants whose presence may be more significantly affected by differences in ethnic specificity, sample handling protocols, or array technology.

As seen in Figure 5, tumours belonging to the Luminal category (subtype S1) appear to be transcriptionally homogenous on the basis of the CIS. To determine if tumours belonging to this subtype could be further subdivided, the inventors reclustered a larger group of Luminal tumours using a separate set of genes which in a previous report had been shown to be indicative of a tissue's cellular proliferative status (Sorlie et al., 2001).

On the basis of these "proliferation genes", they found that the Luminal tumours could be subdivided into two distinct types, namely, "pure" luminal A and another subtype that they have referred to as a Luminal D subtype (Figure 9a). It is likely that the Luminal A/D subdivision is clinically meaningful, as a reclustering of a more diverse set of tumours on the basis of the "proliferation genes" resulted in two broad subdivisions, one representing clinically aggressive tumours (Basal, ERBB2 and Luminal D), and the other representing tumours that are more clinically tractable (Luminal, Normal/Normal-like) (Figure 9b).

Basal-like : The basal molecular subtype was reported in the Stanford study to be characterized by high levels of two expression signatures - I) markers of the basal mammary epithelia, such as keratin 5 and 17, and II) genes belonging to the 'novel' cluster. Consistent with the Stanford study, the inventors also observed a basal subtype associated with similar expression signatures (bar(S4)), indicating that the basal molecular subtype is also highly robust. In addition, however, they also detected the apparent presence of another subtype (bar (S5)) that was not associated with any of the expression signatures described in the Stanford study.

Normal Breast-like : The 'normal-like' subtype is ssociated with expression of a gene cluster that is also highly expressed in normal breast tissues, and includes genes such as *four and a half LIM domains 1*, *aquaporin 1*, and *alcohol dehydrogenase 2 (class I) beta*. A number of tumors in the inventors' series also clustered with the normal breast tissues and exhibited this expression signature (bar (S6)). Thus, the 'normal-like' molecular subtype can also be considered to be a robust subtype.

ERBB2 + : The Stanford study also defined a final ERBB2 + subtype in which these tumors were characterized by high levels of expression of ERBB2 related genes (column E), intermediate levels of expression of the 'novel' cluster (column B), and absent expression of ER-related genes (column A). A similar ERBB2 + subtype was also clearly present in the inventors' series (bar (S3)). Consistent with the expression data, they also subsequently confirmed that the tumors belonging to this molecular subtype were all ERBB2+ by conventional immunohistochemistry as well.

To summarize, of the 5 molecular subtypes defined by the Stanford study, the inventors clearly detected at least 4 subtypes in their own patient population (luminal A, basal-like, normal breast-like, and ERBB2+). They could not clearly determine if one particular subtype (luminal B/C) was present in their series using the genes in the CIS, and they also detected the potential presence of 2 additional subtypes (ER+ ERBB2+ and ER- Subtype II) which have not been reported before. The finding that that the majority (4/5) of the Stanford molecular subtypes were also clearly detectable in the inventors' study suggests that despite many methodological differences between centres, that molecular subtypes as defined by expression based genomics are indeed remarkably robust and conserved between different patient populations.

Ductal Carcinoma in situ (DCIS) Cancers Express The Hallmark Expression Signatures of Invasive Cancer Molecular Subtypes

The previous results indicate that molecularly similar subtypes of breast cancer can indeed occur and be detected across distinct ethnic populations. One limitation of these studies, however, is that it is often very difficult to profile the same cancer over an extended period of time. As such, one question that is often raised is whether these molecular variants represent subtypes that are truly distinct biological entities, or whether they simply reflect a single or a few subtypes in different stages of evolution. Since these two different theories, referred to as the 'distinct origins' and the 'evolutionary' hypotheses respectively (Figure 6e), have different implications for clinical diagnosis and subsequent staging and monitoring, it is thus important to determine which of these proposed mechanisms is the case for breast cancer. Unfortunately, it is not possible to distinguish between these two models by only studying invasive cancers that have been sampled at a single point in time, as both hypotheses would be expected to produce results similar to that shown in Figure 5.

In conventional histopathology, ductal carcinoma-in-situ (or DCIS) has long been recognised as the major precursor to invasive breast cancer, and likely represents the earliest morphologically detectable malignant non-invasive breast lesion. Despite their malignant status, however, DCIS cancers are also distinct from invasive cancers in a number of respects. Clinically, DCIS cancers are treated differently from invasive cancers (DCIS cases are primarily treated with surgery with or without adjuvent radiotherapy) (Harris et al., 1997), and DCIS and invasive cancers also differ substantially in their distribution of specific cancer types (Barnes et al., 1992; Tan et al., 2002). Differences such as these raise the possibility that while DCIS cases are malignant, they may also be molecularly distinct in some respects from more advanced invasive cancers. The inventors reasoned that the 'distinct origins' and 'evolutionary' hypotheses could be tested by profiling a series of DCIS cancers and comparing their profiles to their invasive counterparts. Each hypothesis carries different predictions. If the 'distinct origins' hypothesis is true, then the DCIS cancers, representing 'early' cancers, should express many, if not all, of the hallmark expression signatures associated with their more mature invasive counterparts. Alternatively, if the 'evolutionary' hypothesis is correct, then one might expect that the DCIS profiles to be more closely similar to one another than to their invasive counterparts. The inventors obtained 12 DCIS tissue samples whose histopathological status was confirmed by a pathologist both using conventional H & E staining as well as frozen cryosections of the actual sample that was processed (Figure 2a and b).

Expression profiles of the DCIS samples were then generated and compared to their invasive counterparts. Using the CIS as a starting dataset, the inventors found that the DCIS samples segregated amongst the various invasive cancer samples into distinct categories. Specifically, 5 DCIS samples segregated into the Luminal subtype, 4 into the ER-/ERBB2 + subtype, 2 into the ER +/ ERBB2+ subtype, and 1 into the 'normal breastlike' subtype. Importantly, within each subtype, each of the DCIS cancers was found to robustly express the hallmark expression signatures of its particular molecular group. Interestingly, no DCIS samples were found to cluster within the basal or ER- subtype II molecular subtypes, which is consistent with previously proposed theories that these subtypes may develop without a (or possess an extremely transient) DCIS component (Barnes et al., 1992). These results suggest that distinct breast cancer molecular subtypes are present even at the DCIS stage of breast cancer tumorigenesis, supporting the hypothesis that the subtypes represent truly distinct biological entities, possibly arising via different tumorigenic pathways (the 'distinct origins' hypothesis).

Genes Associated with the Normal/DCIS/Invasive Cancer Transitions Implicate Disregulation of Wnt Signaling as a Common Early Event in Breast Tumorigenesis and that Luminal A and ERBB2+ Cancers Exhibit Similar Invasion Programs

Mammary tumorigenesis can be broadly divided into two main steps : First, normal breast epithelial tissue is transformed to a malignant state via the concerted deregulation of various cellular pathways (Hahn and Weinberg, 2002). Second, to progress to an invasive cancer, several additional biological subprograms also have to be further executed, including penetration of the surrounding basement membrane, invasion of the cancer into the adjacent normal stroma, and angiogenic recruitment of endothelial vessels for tumor nourishment and maintenance (Hanahan and Weinberg, 2000). Given the molecular heterogeneity of breast cancer, one important question in the field is the extent to which the genetic programs that control these two key steps are subtype specific or commonly shared among all breast cancer subtypes.

To identify genes whose expression level was significantly different between normal breast tissues, DCIS cancers, and their invasive counterparts, the inventors used significance analysis of microarrays (SAM), a robust statistical methodology that has been used in previous reports to identify significantly regulated genes (Tusher et al., 2001). They concentrated on studying the luminal and ERBB2+ cancers, as most of the DCIS samples in their study belonged to these two molecular subtypes. First, they tested and confirmed the hypothesis that DCIS cancers, despite expressing many of the hallmarks of invasive cancers, are nevertheless still transcriptionally distinct from invasive cancers. The inventors compared 5 luminal DCIS cancers to 5 luminal invasive cancers, and determined that there existed 222 genes that were significantly regulated using a 2-fold cut-off criterion and a false-discovery rate (FDR) of 5%. In contrast, a control analysis comparing only invasive luminal A cancers which had been randomly distributed into 2 groups failed to identify any significantly regulated genes under these stringent conditions. A similar result was also obtained for DCIS and invasive cancers belonging to the ERBB2+ subtype (data not shown), indicating that significant transcriptional differences exist between DCIS and invasive cancers belonging to both the Luminal A and ERBB2+ subtypes.

SAM was then used to identify genes that were significantly regulated during either the normal/DCIS and DCIS/invasive transitions for both the luminal A and ERBB2 molecular subtypes (FDR = 5%). The results are summarized in Figure 8a. In total, for the luminal A subtype, a greater number of genes were significantly down-regulated during the normal/DCIS transition than upregulated (705 genes down vs 245 genes up), while for the DCIS/Invasive transition more genes were significantly increased in expression than decreased (56 genes down vs 277 genes up). Similarly, for the ERBB2 subtype, 367 genes were significantly downregulated and 275 genes upregulated during the normal/DCIS transition, while 113 genes were downregulated and 294 genes upregulated during the transition from DCIS to invasive cancer.

The following provides an outline as to how the genesets of Table 4, 5, 6 and 7 were determined.

A "Genetic Identifier" that can Distinguish between a normal vs Tumour Breast Sample

Methodology :

Data set: 95 Breast Tissue Samples (11 Normal and 84 Tumors)

Step 1: The data for each sample was normalized by median centering each expression profile around 5000 flouresence units (the Genechip technology measures expression abundance of each gene in terms of flouresence units, from 0 to 65535)

Step 2: An intensity filter was applied such that only genes with intensity values in the range of 200 to 100,000 were retained

Step 3: A 'Valid value' filter was applied such that genes that were at least 70% present (ie above a minimum threshold value, usually about 200) in either normals or tumors or both were retained chosen

Step 4: A statistical T-test was performed to select genes that were differentially expressed in normal vs tumors at a confidence level of $p < 0.00001$. This resulted in the selection of 507 genes

Step 5: Of the 507 genes, a high fold change filter was applied to select genes that exhibited large differences in expression between normal and tumor samples (2.5-fold and above). This resulted in the identification of 49 genes (up in tumors) and 81 genes (up in normals) respectively. These genes are listed in Table 4a.

Step 6: The 130 (49 and 81) genes were ranked using support vector machine gene ranking in order to rank genes in the order of their importance in being able to assign an unknown breast sample to either a tumor or normal group. This was done to arrive at a small subset of genes that can accurately predict normal from tumors. Top 32 genes gave close to 1% misclassification. The results are given in Table 4b.

Step 7: The 32 geneset was tested for its predictive accuracy in the classification of normal vs tumor samples, using leave-one-out cross-validation (LVO CV) testing. No misclassifications were observed.

Support Vector Machine (SVM) Gene Ranking

This approach is used to rank the genes in a dataset according to their importance in being able to assign an unknown sample to a particular group. Typically, the samples in the dataset are divided into a (75%) training and (25%) test set. A maximum margin hyperplane separating the two classes (eg ER+ vs ER-) is calculated for the training set.

Assuming 'm' genes are present in the set, the equation of maximum margin hyperplane is

$$H = W_1 * G_1 + W_2 * G_2 + \ldots\ldots. + W_i * G_i + \ldots\ldots + W_m * G_m$$

Where W_i's are the weights and G_i's refer to the variables (genes).

Using the genes corresponding to various top 'N' weights (weight is indicator of importance of gene in classification) the class of all samples in the test set is predicted. The prediction rules are built for varying sets of top N genes. The above procedure is repeated 100 times and the gene ranks and misclassification rates are averaged.

"Genetic Identifiers" that can Predict the Estrogen Receptor Status and the ERBB2 Receptor Status of a Breast Tumour Sample

Methodology :

Data set: 55 invasive breast tumor samples. The individual tumors were assigned to the following groups on the basis of IHC (immunohistochemistry):

a) Estrogen receptor (ER) status: 35 ER positive and 20 ER negative samples

b) c-erbB-2 (ERBB2) status: 21 ERBB2 positive and 34 ERBB2 negative samples.

Step 1: Gene selection to identify genes that are differentially expressed between a) ER+ vs ER- tumors, and b) ERBB2+ vs ERBB2- samples. Three independent gene selection techniques were used :

- Significance Analysis of Microarrays (SAM), a statistical technique that uses random permutations of the expression data to estimate the 'false discovery rate', ie the chance at which a particular gene will be falsely called as being differentially expressed (Tusher et al., 2001). The genes are then ranked by their "relative difference", which is similar to the ranking used in Step 6, above. The top 100 significant genes were selected.

- A signal to noise (S2N) strategy was used to rank genes based on their correlation with the class distinction (either ER+/ER- or ERBB2+/ERBB2-) (Golub et al., 1999). The top 100 genes were selected.
- A support vector machine (SVM) ranking strategy was used to rank the genes according to their importance in assigning a breast tumor sample to the correct class (see below). The optimal gene set (with highest accuracy) was selected.

Step 2: Common Gene Set (**CGS**): The genes from the 3 independent analysis were pooled, and the common genes selected by all three methods were selected. Hence these genes are method-independent and sufficiently robust to be used as a 'genetic identifier' to predict either the ER or ERBB2 status of a breast tumor sample.

Result:

- For ER classification, the CGS contains 25 unique genes (18 up, 7 down regulated)
- For ERBB2 classification, the CGS contains 26 unique genes (19 up, 7 down regulated)

The genes belonging to each CGS are listed in Table 5. Finally, the accuracy of each CGS for tumor classification was assessed using LVO CV testing. The classification algorithm used was a Support Vector Machine (SVM). Average cross validation error rate = 7.286 % for ER classification (overall accuracy 92%), and 6.26% for ERBB2 classification (overall accuracy 93%).

"Genetic Identifiers" that can Predict the Molecular Subtype of a Breast Tumour Sample

Methodology

Data set : Expression Profiles for tumors belonging to the various subtypes were generated using Affymetrix U133A Genechips. The hallmark expression signatures that characterize each subtype are described above.

a) Luminal (19)
b) ERBB2 (19)
c) Basal (7)
d) ER negative type 2 (5)
e) Normal and Normal like (12)

A. Identification of a Minimal Geneset for Classification Using a One-vs-All Support Vector Machine Approach

Step 1: The data for each sample was normalized by median centering each expression profile around 1000 flouresence units (the Genechip technology measures expression abundance of each gene in terms of flouresence units, from 0 to 65535)

Step 2: A 'Valid value' filter was applied such that genes that were at least 70% present (ie above a minimum threshold value, usually about 200) across all samples were chosen

Step 3: Five different data sets were created are by leaving one of the above-mentioned groups out and combining the four remaining groups (ie 'One-vs-all').

Dataset	Description
1	Luminal (19) vs Rest (43)
2	ERBB2 (19) vs Rest (43)
3	Basal (7) vs Rest (55)
4	ER negative type 2 (5) vs Rest (57)
5	Normal and Normal like (12) vs Rest (50)

Step 4: For each of the 5 datasets, genes were selected that exhibited a minimum 2 fold change between groups (Ratio of means was used to calculate the fold change between two groups).

The results are as follows

Dataset	Description	Differentially regulated (2 fold)
1	Luminal (19) vs Rest (43)	116
2	ERBB2 (19) vs Rest (43)	46
3	Basal (7) vs Rest (55)	318
4	ER negative type 2 (5) vs Rest (57)	309
5	Normal and Normal like (12) vs Rest (50)	188

Step 5: A support vector machine gene ranking analysis was performed for each of the five datasets to rank genes in the order of their importance in assigning an unknown breast sample to its appropriate class (e.g. ER or ERBB2 status, see above).

For datasets 1,3,4, and 5, a geneset was selected that yielded a 3% misclassification rate. In case the case of dataset 2 (ERBB2 vs rest), the use of all 46 genes gave a minimum of 9.7 error rate. Hence, all 46 were used in the predictor set. The predictor sets are shown in Table 6.

Dataset	Description	Differentially regulated (2 fold)	Top 'N' genes	Error rate
1	Luminal (19) vs Rest (43)	116	35	3
2	ERBB2 (19) vs Rest (43)	46	46	9.7
3	Basal (7) vs Rest (55)	318	20	3
4	ER negative type 2 (5) vs Rest (57)	294	111	3
5	Normal and Normal like (12) vs Rest (50)	188	50	3

Step 6: The samples were all combined into one dataset and one vs all cross-validation analysis was carried out using the various predictor sets. 100 independent iterations of 75:25 (training:test) random splits were used, resulting in an overall cross validation error rate of 5.25% (Overall accuracy 94%).

B. Identification of a Minimal Geneset for Classification Using a Genetic Algorithm/Maximum Likelihood Discriminant (GA/MLHD) Approach

The GA/MLHD approach is a different classification algorithm (Ooi & Tan, 2003) that serves as an alternative to the OVA SVM described in A.

Step 1: Samples were broken down into the following classes:

Class	No. of samples
ER- subtype II	5
ERBB2+	19
Normal and Normal-like	12
Luminal	19
Basal	7

A truncated dataset of 1000 genes was then established by selecting genes that exhibited the largest standard deviation (SD) across all the samples.

Step 2: 24 runs of the GA/MLHD algorithm were performed on the 62 breast cancer samples based on the class distinction described in Table 4. The accuracy of the predictor sets selected by the GA/MLHD algorithm were assessed by cross-validation and independent test studies.

Details of GA/MLHD properties:

(a) Crossover rates: 0.7, 0.8, 0.9, 1.0.

(b) Mutation rates: 0.0005, 0.001, 0.002, 0.0025, 0.005, 0.01

(c) Uniform crossover

(d) Selection: stochastic uniform sampling

(e) Predictor set size range: R_{min} = 1 and R_{max} = 80.

30 optimal predictor sets with sizes ranging from 13 to 17 genes per predictor set were obtained. Each predictor set was associated with a classification accuracy of 1 error out of 62 samples. (error rate: 1.61%, overall classification accuracy 98%). 10 out of the 30 predictor sets wrongly classified the Luminal-A sample 980221T as a Normal sample. For the other 20 predictor sets, 19 misclassified the ERBB2+ sample 990262T as a ER- subtype II sample, while 1 predictor set wrongly classified the same 990262T sample as a Basal-type sample. Two of the optimal predictor sets are displayed in Table 6b.

Identification of a Luminal D Subclass in the Asian Breast Cancer Population

Previous breast cancer expression profiling studies done on primarily Caucasian populations revealed the existence of a 'luminal' subtype characterized by the high expression of estrogen-receptor related genes such as ESR1, GATA3, and LIV-1. Further, these 'luminal' cancers could be further subdivided into at least 2 further subtypes : Luminal A and Luminal B/C. While Luminal A tumors express very high levels of ER related genes, Luminal B/C cancers express intermediate levels of the ER gene cluster. Furthermore, luminal C tumors also express high levels of a 'novel' gene cluster. Luminal B/C tumors were found to exhibit a worse clinical prognosis than Luminal A tumors, arguing that these subtypes are indeed clinically relevant.

A similar study on breast cancers derived from Chinese patients performed in Singapore confirmed that the luminal A subtype is also present in the Asian patient population. However, the luminal B/C subtype was not detected. The reasons behind this difference may be due to methodological differences between the two studies or true differences in patient population.

A careful inspection of the original Caucasian study by the inventors subsequently revealed that Luminal C tumors are also associated with high levels of a gene cluster whose members are involved in cellular proliferation. In contrast, this 'proliferation cluster' is lowly expressed in Luminal A tumors. The high expression of genes in the 'proliferation cluster' may functionally contribute to the worse clinical prognosis associated with Luminal C tumors, as this high expression levels of this cluster is also seen in tumors belonging to the clinically aggressive ERBB2+ and basal (ER-) subtypes as well. Thus, although a luminal B/C subtype was not observed in the Asian breast cancer population, the inventors hypothesized that the genes in this 'proliferation' cluster could also be used to subdivide the previously homogenous Luminal A tumors found in the Asian population into distinct luminal subtypes.

Results

Identification of 'proliferation cluster' linked-genes on the Affymetrix U133A Genechip

In the inventor's study, the expression profiles of several breast tumors were obtained using commercially available Affymetrix U133A Genechips. Genes corresponding to the original 'proliferation' cluster members were then selected from the Genechip. Of the 65 genes comprising the original 'proliferation cluster', the inventors determined at 36 (55%) were also present on the Genechip array.

Discovery of a 'Luminal D' Subtype in the Asian Luminal Tumor Population

The inventors then used this 36-geneset to recluster a group of tumors which in their previous analysis had been homogenously assigned to the Luminal A subtype. As seen in Figure 1, the 36-geneset strikingly divided the tumors into two broad groups chracterized by low and high levels of expression of the 36-geneset respectively. The former group is from henceforth referred to as the true 'luminal A' subtype, while the latter group is referred to as 'luminal D', as its expression profile is distinct from previously identified subtypes.

High levels of expression of the 36-geneset is also observed in other aggressive tumor subtypes

To determine if Luminal D tumors are also more clinically aggressive than Luminal A tumors, the inventors then determined if high expression levels of this cluster was also observed in aggressive tumors subtypes by reclustering a larger series of their tumors using only the 36-gene 'proliferation cluster'. As seen in Figure 2, Luminal D tumors intermixed with tumors of the ERBB2+ and Basal subtypes, while Luminal A tumors mixed with the normal and 'normal-like' tumors. This result suggests that the Luminal D tumors may share certain hallmarks of more highly aggressive tumors, and that the Luminal D subtype may be clinically relevant.

A 'Genetic Identifier' for the Luminal D Subtype

The inventors then proceeded to develop a 'genetic identifier' for the Luminal D subtype. In this strategy, the 'genetic identifier' should only be applied to a tumor that has previously been characterized as Luminal in nature, for example by the other 'genetic identifiers' shown in Tables 5 and 6.

Step 1: A series of expression profiles for 19 tumors which had been previously characterized as Luminal A were normalized by median centering each expression profile around 1000 flouresence units.

Step 2: A 'Valid value' filter was applied such that genes that were at least 70% present (ie above a minimum threshold value, usually about 200) across all samples were chosen

Step 3: To divide the samples in a more robust fashion, a Principal Component Analysis (PCA) was then used to ascertain the Luminal A and D subgroups using the 36 proliferation geneset (Figure 3).

Step 4: Using the Luminal A (12 samples) vs. Luminal D (7 samples) groupings, genes were selected from the entire expression profile that exhibited a minimum 2 fold change between the two groups (Ratio of means was used to calculate the fold change between two groups). 111 such genes were identified in this analysis.

Step 5: A SVM gene ranking analysis was then performed for the 111-gene dataset to rank genes in the order of their importance in assigning a luminal breast cancer sample into either the Luminal A or Luminal D subtypes. The top 45 genes gave lowest error rate (about 12%). 18 genes were up regulated in Luminal D and 27 were down regulated in luminal D. The genes are depicted in Table 7.

Step 6: The accuracy of the 45-gene Genetic identifier was then assesed using leave one out cross validation. No misclassifications were observed.

Discussion

One outstanding challenge of the post-genomic era is to translate the huge amounts of raw sequence data generated by various genome sequencing projects into applications that improve healthcare and the treatment of disease. One area which could be revolutionised by the availability of these new resources is in the field of molecular diagnostics, where the pathologic classification of a tissue, in complementation to conventional histopathology, is also based upon a set of informative molecular markers. Importantly, one advantage of the molecular approach is that the resolving power of classification schemes based upon molecular data can be sufficiently sensitive to detect clinically relevant disease subtypes that have currently eluded traditional light microscropy approaches (Ash et al., 2000, Bittner et al., 2000).

However, before the potential of molecular diagnostics can fully realized, a number of challenges must be met and overcome. Firstly, for many common diseases, key informative genes that are able to discriminate between the relevant disease sub-classes in question must be identified. Secondly, in order to be feasibly utilized as part of a clinical assay, these genes must be 'pared' down to a minimal set ('genetic identifiers') that collectively still delivers high predictive accuracy. Thirdly, because the clinical behaviour of many diseases can vary extensively amongst different ethnic groups and populations, it will be necessary to define appropriate limits of use of these 'genetic identifiers' for specific patient populations.

To address these issues, the inventors have embarked upon a large-scale expression profiling project of breast tissues derived from Asian patients. _Previous reports have primarily focused on using samples derived from patients of primarily Caucasian origin (Perou et al., 2000, Gruvberger et al., 2000, Hedenfalk et al., 2000), and it is essential to determine if findings obtained from these studies will be applicable to other ethnic populations. This is especially so given the epidemiological and clinical differences in breast cancer between these distinct ethnic groups. In Caucasian populations, the majority of breast cancers tend to occur in post-menopausal women. However, in Singapore and Japan, the absolute number of breast cancer cases per year is roughly 1/3 that of the US and the incidence of breast cancer in these populations is bi-modal - the first peak, representing the majority of breast cancers, occurs in pre-menopausal women occurs at around the age of 40 (Chia et al., 2000). This first peak is then followed by a second peak at about age 55-60. The earlier incidence of breast cancer in Asian populations is unlikely to be due to earlier detection, as breast cancer screening programs in these countries are still relatively novel compared to Western countries. To explain these observations, one possibility may be that the breast cancers observed in these groups may represent distinct heterogenous subtypes arising from specific genetic or environmental differences. For example, it is known that the levels of estrogen and progesterone in Chinese women tend to be substantially lower than in Caucasians (Lippman, 1998).

To ensure maximal diversity in the repertoire of expression profiles used in the inventors' analysis, the inventors selected samples derived from patients from a wide variety of demographic and clinical backgrounds, as well as tumours of varying grades and appearances. First, the inventors identified a 'genetic identifier' in breast cancer for what is perhaps the most basic distinction of clinical utility -

i.e. distinguishing if a given sample is 'normal' or 'malignant'. Although this distinction can be currently made by a qualified pathologist using conventional histopathology, the availability of such a molecular assay would still be of use in clinical settings where rapid diagnosis is required, or when a pathologist may not be readily available. By focusing on highly reproducible 'outlier' genes in both normal and tumour datasets, the inventors identified a minimal set of 20 genes that is apparently able to accurately predict if an unknown breast sample is normal or malignant in both a training set and naïve test set of comparable sample quantity. In addition, using principal component analysis, they were able to show that at the expression profiles of normal breast samples appears to be far less varied than their corresponding tumour profiles. In the field of breast cancer research, there are surprisingly relatively few reports in the literature that have directly addressed the question of distinguishing between normal and tumour tissues using the relatively unbiased manner afforded by the DNA microarray approach. In one major study, it was found that that the expression profiles of normal breast tissues were sufficiently similar for them to co-segregate with each other using an unsupervised clustering methodology (Perou et al., 2000). However, in that report, the investigators also found that the normal samples, rather than segregating as an independent branch distinct from the tumour samples, instead segregated within a broad tumour class originating from mammary epithelial cells of 'basal' or 'myoepithelial' origin. This result, most likely due to the similarity of genes that are expressed in normal tissues and tumours of this subclass, illustrates that it may not be trivial to use purely unsupervised methodologies to discriminate between normal and tumour breast tissues. However, while this appears to be an issue for breast cancer genomics, it may not apply to other tissue types. For example, it appears that unsupervised clustering is able to discriminate between normal and malignant colon samples (Alon et al., 1999). One reason for this may be that colon tumours, which primarily arise from disruption of the APC/β-catenin pathway, may be genetically more uniform than breast tumours.

The genes involved in the 20-gene 'genetic identifier' belong to many different categories. Genes such as apolipoprotein D are well-known terminal differentiation genes in breast biology, while MAGED2 was previously isolated as a gene that is overexpressed in primary breast tumours, but not in normal mammary tissue or breast cancer cell lines (Kurt et al., 2000). Another gene, ITA3, which produces the alpha-3 subunit of the alpha-3/beta-1 integrin, has been shown to be associated with mammary tumour metastasis (Morini et al., 2000). The CAV1 protein, which links integrin signaling to the Ras/ERK pathway, has also previously been identified as a potential tumour suppressor gene (Wary et al., 1998, Weichen et al., 2001), which may explain its expression in normal breast tissues but not tumours. In addition to genes with known roles in breast and tumour biology, other intriguing genes were identified whose role in tumourgenesis is unclear or not known. For example, thrombin, best known for its role in the coagulation cascade, has recently been shown to inhibit tumour cell growth, which may explain its expression in normal but not tumour breast samples (Huang et al., 2000).

Another example is the human homolog of the *S. cerevisiae* PWP2 gene, which in yeast plays an essential role in cell growth and separation (Shafaatian et al., 1996).

To gain insights into the diversity of breast cancer molecular subtypes in the Asian population, the inventors then generated and analyzed a series of expression profiles of both invasive breast cancers and DCIS cancers. The aim of this work was to attempt to validate the molecular subtyping scheme defined in the Stanford study using another breast cancer expression dataset. By comparing their expression profiles to previously published studies performed using patient samples of primarily Caucasian origin, they found that the majority of molecular subtypes and hallmark expression signatures were robustly conserved between the two series. Although a similar validation study has recently been reported for prostate cancer (Rhodes et al., 2002), this report is the first time such a comparative analysis has been performed for breast cancer. The conservation of molecular subtypes between the two populations is all the more remarkable when one considers the many methodological differences existing between the studies. For example, one finding of interest was the inventors' ability to detect similar subtypes in both series despite the differences in array technology platform. This result is significant as there is currently conflicting data in the field regarding the feasibility of integrating data from different genomic expression technologies. For example, in Rhodes et al., (2002), it was reported that prostate cancer expression data from spotted cDNA arrays yielded similar data to oligonucleotide arrays.

In contrast, another recent report comparing the expression profiles of cell lines as measured by spotted and oligonucleotide arrays reported a very poor correlation between the studies (Kuo et al., 2002). The inventors' results suggest that data from different technology platforms can indeed be compared, so long as the subtype distinctions in question are fairly robust in nature.The inventors' results also suggest that despite the epidemiological differences in breast cancer between the Asian and Caucasian population (see beginning of Discussion) , that breast cancers between the ethnic groups are to a first approximation highly molecularly similar.

The inventors also found that DCIS cancers robustly express many subtype-specific gene expression signatures, suggesting that these molecular subtypes can be discerned even at this pre-invasive stage. Thus, it is unlikely that these subtypes represent an evolving cancer class, but are distinct biological entities that may posses different tumorigenic origins. Despite the expression of subtype-specific expression signatures in DCIS cancers (as reported in this study), there is other evidence in the field that DCIS cancers may be distinct from invasive cancers. For example, previous retrospective reports have shown that the majority of low nuclear grade DCIS tumors undergo a long clinical evolution to invasive cancer (Page et al., 1982; Betsill et al., 1978; and Rosen et al., 1980), suggesting that additional genetic events must occur before they become invasive. In addition, histopathological studies have found that there is a considerable difference in the histopathological distribution of tumor types in DCIS cancers vs invasive cancers, with ERBB2+ cancers being much more highly represented in DCIS compared to invasive cases (Barnes et al., 1992). It has been unclear, however, if this observation should be interpreted to mean that that the ER-ERBB2- cancers lack a DCIS component, or if the ERBB2+ cancers will eventually evolve to a ERBB2- state. The distinctive segregation of the DCIS cancers in the inventors' series suggests that the former is true, since the ERBB2+ cancers already express many ERBB2+ invasive hallmarks.

Finally, by integrating the expression profiles of normal, DCIS, and invasive cancers belonging to the luminal A and ERBB2+ subtypes, the inventors were able to define sets of genes which were regulated in a common and subtype-specific manner during the normal, DCIS, and invasive cancer transitions. Although the results of these analyses clearly need to be supported by further experimental work before any definitive conclusions can be made, there were a number of intriguing observations. The inventors found that a number of components of the Wnt signaling pathway were commonly regulated during the transition from normal -> DCIS for both subtypes, implicating deregulation of Wnt signaling as an important common event in breast cancer carcinogenesis. Although previous reports have reported the involvement of the Wnt pathway in human breast cancer carcinogenesis (Smalley et al., 2001), it has been less clear if this is an early or late event. The inventors' results suggest the former possibility is more likely. Secondly, the remarkable commonality of genes regulated from the DCIS to the invasive stage between the two subtypes suggests that many of the genetic processes that underlie cellular invasion, desmoplastic reaction, stromal remodeling etc, may be fairly general and shared across different breast cancer subtypes. Finally, the inventors' results also suggest that both cancer subtypes may be highly metabolically distinctive, with ERBB2+ tumors having a greater reliance on ionic-related processes, while Luminal A tumors may be under a state of chronic metabolic stress. These results are extremely important, for example, the increased metabolic load of Luminal A tumors may explain why ER+ tumors are more radiosensitive than ER- tumors (Villalobos et al., 1996), and calcium signaling may play a role in tumor cell motility controlled by the ERBB2+ receptor (Feldner and Brandt (2002).

References

Alon, U., N. Barkai, D. A. Notterman, K. Gish, S. Ybarra, D. Mack, and A. J. Levine (1999) Broad patterns of gene expression revealed by clustering analysis of tumour and normal colon tissues probed by oligonucleotide arrays. Proc Natl Acad Sci 96,

Ash, A. A., M. B. Eisen, R. E. Davis, C. Ma, I. S. Lossos, A. Rosenwald, J. C. Boldrick, H. Sabet, T. Truc, Y. Xin, J. I. Powell, L. Yang, G. E. Marti, T. Moore, J. Hudson, L. Lisheng, D. B. Lewis, R. Tibshirani, G. Sherlock, W. C. Chan, T. C. Greiner, D. D. Weisenburger, J. O. Armitage, R. Warnke, R. Levy, W. Wilson, M. R. Grever, J. C. Byrd, D. Botstein, P. O. Brown, and L. M. Staudt (2000) Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 403, 503-511

Barnes, D. M., J. Bartkova, R. S. Champlejon, W. J. Gullick, P. J. Smith, and R. R. Millis (1992) Overexpression of c-erbB2 Oncoprotein : Why does this occur more frequently in ductal carcinoma in situ than in invasive mammary carcinoma and is this of prognostic significance? Eur J Cancer 28, 644-648

Betsill, W. L. J., P. P. Rosen, P. H. Lieberman, and G. F. Robbins (1978) Intraductal carcinoma. Long-term follow-up after treatment by biopsy alone. JAMA 239, 1863-1867

Bittner, M., P. Meltzer, Y. Chen, Y. Jiang, E. Seftor, M. Hendeix, M. Radmacher, R. Simon, Z. Yakhini, A. Ben-Dor, N. Sampas, E. Dougherty, E. Wang, F. Marincola, C. Gooden, J.

Lueders, A. Glatfelter, P. Pollock, J. Carpten, E. Gillanders, D. Leja, K. Dietrich, C. Beaudry, M. Berens, D. Alberts, V. Sondak, N. Hayward, and J. Trent (2000) Molecular classification of cutaneous malignant melenoma by gene expression profiling. Nature 406, 536-540

Chia, K. S., A. Seow, H. P. Lee, and K. Shanmugaratnam (2000) Cancer Incidence in Singapore, 1993-1997. In (Singapore Cancer Registry)

DeRisi, J. L., V. R. Iyer, and P. O. Brown (1997) Exploring the Metabolic and Genetic Control of Gene Expression on a Genomic Scale. Science 278, 680-686

Eisen, M. B., P. T. Spellman, P. O. Brown, and D. Botstein (1998) Cluster analysis and display of genome-wide expression patterns. Proc Natl Acad Sci 95, 14863-14868

Feldner, J. C. and B. H. Brandt (2002) Cancer cell motility - on the road from c-erbB-2 receptor steered signaling to actin reorganization. Exp Cell Res 272, 93-108

Giuliano, A. E. (1998) Breast. In Current Medical Diagnosis and Treatment, 37, Ed. Tierney, L. M.S. J. McPhee and M. A. Papadakis (Appleton and Lange, Stamford) 666-690

Golob, T. R., D. K. Slonim, P. Tamayo, C. Huard, J. P. Gaasenbeek, H. Coller, M. L. Loh, J. R. Downling, M. A. Caligiuri, C. D. Bloomfield, and E. S. Lander (1999) Molecular Classification of Cancer : Class Discovery and Class Prediction by Gene Expression Monitoring. Science 286, 531-537

Gruvberger, S., M. Ringner, Y. Chen, S. Panavally, L. H. Saal, A. Borg, M. Ferno, C. Peterson, and P. Meltzer (2001) Estrogen Receptor Status in Breast Cancer is Associated with Remarkably Distinct Gene Expression Patterns. Cancer Research 61, 5979-5984

Hahn, W. C. and R. A. Weinberg (2002) Rules for making human tumor cells. N Engl J Med 347, 1593-1603

Harris, J. R., M. Morrow, and L. Norton (1997) Malignant Tumors of the Breast. In Cancer:Principles and Practice of Oncology, Ed. Devita, V. T.S. Hellman and S. A. Rosenberg (Lippincott-Raven, Philadelphia/New York).

Hanahan, D. and R. A. Weinberg (2000) The hallmarks of Cancer. Cell 100, 57-70

Hedenfalk, I., D. Duggan, Y. Chen, M. Radmacher, M. Bittner, R. Simon, P. Meltzer, B. Gusterson, M. Esteller, O. P. Kallioniemi, M. Wilfond, A. Borg, and J. Trent (2001) Gene Expression Profiles in Hereditary Breast Cancer. NEJM 344, 539-548

Huang, Y., J. Li, and S. Karpatkin (2000) Thrombin inhibits tumour cell growth in association with up-regulation of p21(waf1/cip1) and Caspases via a p53-independent, STAT-1-dependent pathway. J. Biol. Chem. 275, 6462-6488

Khan, J., J. s. Wei, M. Ringner, L. H. Saal, M. Ladanyi, F. Westermann, F. Berthold, M. Schwab, C. R. Antonescu, C. Peterson, and P. S. Meltzer (2001) Classification and diagnostic prediction of cancers using gene expression profiling and artificial neural networks. Nature Med 7, 673-679

Kurt, R. A., W. J. Urba, and D. D. Schoof (2000) Isolation of genes overexpressed in freshly isolated breast cancer specimens. Breast Cancer Res. Treat. 59, 41-48

Kuo, W. P., T. K. Jenssen, A. J. Butte, L. O. Machado, and I. S. Kohane (2002) Analysis of measured mRNA measurements from two different microarray technologies. Bioinformatics 18, 405-412

Kuukasjarvi, T., J. Kononen, H. Helin, K. Holli, and J. Isola (1996) Loss of estrogen receptor in recurrent breast cancer is asociated with poor response to endocrine therapy. J. Clin. Oncol. 14, 2584-2589

Lippman (1998) Breast Cancer. In Harrison's Principles of Internal Medicine, 91, Ed. Fauci, A. S.E. BraunwaldK. J. IsselbacherJ. D. WilsonJ. B. MartinD. L. KasperS. L. Hauser and D. L. Longo (McGraw-Hill, New York) 562-568

Morini, M., M. Mottolese, N. Ferrari, G. Ghiorzo, S. Buglioni, R. Mortarini, D. M. Noonon, P. G. Natali, and A. Albini (2000) The alpha-3 beta 1 integrin is associated with mammary carcinoma cell metastasis, invation, and gelatinase B (MMP-9) activity. Int J Cancer 87, 336-342

Ooi C.H. and Patrick Tan (2003). Genetic algorithms applied to multi-class prediction for the analysis of gene expression data. Bioinformatics. 19, 37-44.

Page, D., W. Dupont, L. Rogers, and M. Landenberger (1982) Intraductal carcinoma of the breast: follow-up after biopsy only. Cancer 49, 751-758.

Parl, F. F. (2000) Estrogens, Estrogen Receptor, and Breast Cancer. (IOS Press)

Perou, C. M., S. S. Jeffrey, M. van de Rijn, C. A. Rees, M. B. Eisen, D. T. Ross, A. Pergemenschikov, C. F. Williams, S. X. Zhu, J. C. F. Lee, D. Lashkari, D. Shalon, P. O. Brown, and D. Botstein (1999) Distinctive gene expression patterns in human mammary epithelial cells and breast cancers. Proc Natl Acad Sci 96, 9212-9217

Perou, C. M., T. Sorlie, M. B. Eisen, v. d. R. M., S. S. Jeffrey, C. A. Rees, J. R. Pollack, D. T. Ross, H. Johnsen, L. A. Akslen, O. Fluge, A. Pergamenschikov, C. Williams, S. X. Zhu, P. E. Lonning, A. L. Borresen-Dale, P. O. Brown, and D. Botstein (2000) Molecular Portraits of Human Breast Tumours. Nature 406, 747-752

Rhodes, D. R., T. R. Barrette, M. A. Rubin, D. Ghosh, and A. M. Chinnaiyan (2002) Meta-analysis of Microarrays : Interstudy Validation of Gene Expression Profiles Reveals Pathway Dysregulation in Prostate Cancer. Cancer Research 62, 4427-4433

Rosen, P., D. Braun, and D. Kinne (1980) The clinical significance of pre-invasive breast carcinoma. Cancer 46, 919-925

Shafaatian, R., M. A. Payton, and J. D. Reid (1996) PWP2, a member of the WD-repeat family of proteins, is an essential Saccharomyces cerevisiae gene involved in cell separation. Mol Gen Genet. 252, 101-114

Smalley, M. J. and T. C. Dale (2001) Wnt signaling and mammary tumorigenesis. J Mammary Gland Biol Neoplasia 6, 37-52

Sorlie, T., C. M. Perou, R. Tibshirani, T. Aas, S. Geisler, H. Johnsen, T. Hastie, M. B. Eisen, M. van de Rijn, S. S. Jeffrey, T. Thorsen, H. Quist, J. C. Matese, P. O. Brown, D. Botstein, P. E. Lonning, and A. L. Borresen-Dale (2001) Gene Expression Patterns of Breast Carcinomas Distinguish Tumor Subclasses with Clinical Implications. Proc. Natl. Acad. Sci. 98, 10879-10874

Tan, P. H., K. L. Chuah, G. Chiang, C. Y. Wong, F. Dong, and B. H. Bay (2002) Correlation of p53 and cerbB2 expression and hormonal receptor status with clinicopathological parameters in ductal carcinoma in situ of the breast. Oncology Reports 9, 1081-1086

Tavassoli, F. A. and S. J. Schnitt (1992) Pathology of the Breast. In (Elsevier)

Tusher, V. G., R. Tibshirani, and G. Chu (2001) Significance Analysis of Microarrays Applied to the Ionizing Radiation Response. Proc. Natl. Acad. Sci. 98, 5116-5121

van't Veer, L. J., H. Dai, M. J. van de Vijver, Y. D. He,

A. A. M. Hart, M. Mao, H. L. Peterse, K. van der Kooy, M. J. Marton, A. T. Witteveen, G. J. Schreiber, R. M. Kerkhoven, C. Roberts, P. S. Linsley, R. Bernards, and S. H. Friend (2002) Gene expression profiling predicts clinical outcome of breast cancer. Nature 415, 530-536

Villalobos, M., d. Becerra, M. I. Nunez, M. T. Valenzuela, E. Siles, N. Olea, V. Pedraza, and J. M. Ruiz de Almodovar (1996) Radiosensitivity of human breast cancer cell lines of different hormonal responsiveness. Modulatory effects of oestradiaol. Int J Radiat Biol 70, 161-169

Wang, E., L. D. Miller, G. A. Ohnmacht, E. T. Liu, and F. M. Marincola (2000) High-fidelity mRNA amplification for gene profiling. Nature Biotech. 18, 457-459

Wary, K. K., A. Mariotti, c. Zurzolo, and F. G. Giancotti (1998) A requirement for caveolin-1 and associated kinase Fyn in integrin signaling and anchorage-dependent cell growth. Cell 94, 625-634

Wiechen, K., L. Diatchenko, A. Agoulnik, K. M. Scharff, H. Schober, K. Arlt, B. Zhumabayeva, P. D. Siebert, M. Dietel, R. Schafer, and C. Sers (2001) Caveolin-1 is down-regulated in human ovarian carcinoma and acts as a candidate tumour suppressor gene. Am J Pathol. 159, 1635-1643

Table 1 : Common Genes in Both Normal and Tumour Datasets

NCC ID	Unigene ID	Accession No	GeneName	Annotation
2914401	Hs.151738	NM_004994	MMP9	matrix metalloproteinase 9 (gelatinase B, 92kD gelatinase, 92kD type IV collagenase)
2957001	Hs.50758	BF239180	SMC4L1	SMC4 (structural maintenance of chromosomes 4, yeast)-like 1
3080701	Hs.279009	BF679062	MGP	matrix Gla protein
3080801	Hs.98428	NM_018952	HOXB6	homeo box B6
3082201	Hs.211573	NM_005529	HSPG2	heparan sulfate proteoglycan 2 (perlecan)
3085601	Hs.156110	AW404507	IGKC	immunoglobulin kappa constant
3119301	Hs.78045	NM_001615	ACTG2	actin, gamma 2, smooth muscle, enteric
3174801	Hs.95972	BE892678	SILV	silver (mouse homolog) like
3296301	Hs.153952	AW072424	NT5	5' nucleotidase (CD73)
3390901	Hs.572	X02544	ORM1	orosomucoid 1
3401301	Hs.155421	AA334619	AFP	alpha-fetoprotein
3404301	Hs.25817	AW195430	BTBD2	BTB (POZ) domain containing 2
3437301	Hs.78771	AI525579	PGK1	phosphoglycerate kinase 1
3451301	Hs.56205	AW663903	INSIG1	insulin induced gene 1
3610001	Hs.30743	AI017284	PRAME	preferentially expressed antigen in melanoma
3617301	Hs.10842	AF052578	RAN	RAN, member RAS oncogene family
3619101	Hs.337764	AB038162	NA	trefoil factor 1
3767201	Hs.274184	AF207550	TFE3	transcription factor binding to IGHM enhancer 3
3812201	Hs.914	X03100	AGL	Human mRNA for SB classII histocompatibility antigen alpha-chain
3955201	Hs.19710	H60423	SLC17A2	solute carrier family 17 (sodium phosphate), member 2
4021001	Hs.2055	AA232386	UBE1	ubiquitin-activating enzyme E1

Table 2 : Genes found in the minimal breast cancer genetic identifier

NCC ID	Unigene ID	Accession No	Genename	Annotation	On in Tumour
2920901	Hs.76530	AU121309	F2	coagulation factor II (thrombin)	N
2933601	Hs.278411	AB014509	NCKAP1	NCK-associated protein 1	N
2934801	Hs.79380	AP001753	PWP2H	PWP2 homolog	N
2936101	Hs.1940	AV733563	CRYAB	crystallin, alpha B	N
2987501	Hs.75736	J02611	APOD	apolipoprotein D	N
3041201	Hs.295944	BG621010	TFPI2	tissue factor pathway inhibitor 2	N
3110601	Hs.74034	BG541572	CAV1	caveolin 1, caveolae protein, 22kD	N
3119401	Hs.184411	AL558086	ALB	albumin	N
3143701	Hs.156346	NM_001067	TOP2A	topoisomerase (DNA) II alpha (170kD)	N
3401301	Hs.155421	AA334619	AFP	alpha-fetoprotein	N
2919801	Hs.177766	BE740909	ADPRT	ADP-ribosyltransferase (NAD+; poly (ADP-ribose) polymerase)	Y
2930501	Hs.265829	D01038	ITGA3	integrin, alpha 3 (antigen CD49C, alpha 3 subunit of VLA-3 receptor)	Y
2961201	Hs.4437	AU131942	RPL28	ribosomal protein L28	Y
3048301	Hs.4943	BE891065	MAGED2	hepatocellular carcinoma associated protein; breast cancer associated gene 1	Y

3085601	Hs.156110	AW404507	IGKC	immunoglobulin kappa constant	Y
3119301	Hs.78045	NM_001615	ACTG2	actin, gamma 2, smooth muscle, enteric	Y
3124401	Hs.145279	NM_003011	SET	SET translocation (myeloid leukemia-associated)	Y
3134101	Hs.73885	U88244	HLA-G	HLA-G histocompatibility antigen, class I, G	Y
3193001	Hs.84298	BE741354	CD74	CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated)	Y
3296401	Hs.183601	U70426	RGS16	regulator of G-protein signalling 16	Y

Genes are ordered according to their correlation to the tumour/normal class distinction.

Table 3: Tabulation of expression signatures associated with breast tumor subtypes. Subclasses include Luminal A (L-A_, Luminal B (L-B), Luminal C (L-C_, Basal (Bas), Normal like (Nor), ERBB2 (ERB). Levels of expression are indicated by H (high expression), I (intermediate expression), and A (absent expression).

Expression Signature	Unigene	Tumor subtype					
		L-A	L-B	L-C	Bas	Nor	ERB
Luminal Epithelium		H	I	I	A	A	A
estrogen receptor 1	Hs.1657						
GATA binding protein 3	Hs.169946						
LIV-1	Hs.79136						
Xbox binding protein 1	Hs.149923						
Hepatocyte Nuclear Factor 3 alpha	Hs.299867						
Basal Epithelium		A	A	A	H	H	A
Keratin5	Hs.195850						
Keratin17	Hs.2785						
Laminin gamma 2	Hs.54451						
Fatty acid binding protein 7	Hs.26770						
erbb2 related genes		A	A	A	A	A	H
c-ERB-B2	Hs.323910						
GRB7	Hs.86859						
TIAF1	Hs.75822						
TRAF4	Hs.8375						
Normal breast like		A	A	A	A	H	A
CD36 antigen collagen type 1 receptor	Hs.75613						
Four and a half LIM domain 1	Hs.239069						
vascular adhesion protein 1	Hs.198241						
alcohol dehydrogenase 2 class 1	Hs.4						
Novel		A	A	H	H	A	I
kinesin-like 5 mitotic kinesin-like protein 1	Hs.270845						
putative integral membrane transporter	Hs.296398						
gamma-glutamyl hydrolase conjugase	Hs.78619						
squalene epoxidase	Hs.71465						

Table 4a : Set of 49 Genes Upregulated in Tumors and 81 Genes Upregulated in Normals

Upregulated in tumors

Probe	Gene Description	UniGene	GeneBank	Normal_median	Tumor _median	Fold change (normal/tumor)	P-value
221730_at	collagen, type V, alpha 2	Hs.82985	NM_000393.1	2989.34	22050.38	0.135568639	6.53E-08
205483_s_at	interferon-stimulated protein, 15 kDa	Hs.833	NM_005101.1	3440.12	19587.87	0.175625017	2.89E-09
201422_at	interferon, gamma-inducible protein 30	Hs.14623	NM_006332.1	4216.08	22685.34	0.185850421	5.13E-11
202311_s_at	collagen, type I, alpha 1	Hs.172928	NM_000088.1	2309.8	11583.18	0.199409834	5.47E-08
214290_s_at	H2A histone family, member O	Hs.795	AA451996	8270.53	34668.82	0.238558163	0.000011
204170_s_at	CDC28 protein kinase 2	Hs.83758	NM_001827.1	2364.5	9307.97	0.254029611	2.44E-09
204620_s_at	chondroitin sulfate proteoglycan 2 (versican)	Hs.81800	NM_004385.1	8494.23	31700.6	0.267951711	1.64E-10
201261_x_at	biglycan	Hs.821	BC002416.1	3832.74	14200.24	0.269906706	2.96E-10
221731_x_at	chondroitin sulfate proteoglycan 2 (versican)	Hs.81800	J02814.1	10044.24	36814.75	0.272831949	1.97E-09
203936_s_at	*matrix metalloproteinase 9 (gelatinase B, 92kD gelatinase, 92kD type* IV collagenase)	Hs.151738	NM_004994.1	2908.93	10635.99	0.273498753	1.4E-06
213909_at	Homo sapiens cDNA FLJ12280 fis, clone MAMMA1001744	Hs.288467	AU147799	2270.33	8261.75	0.274800133	2.93E-07
204619_s_at	*chondroitin sulfate proteoglycan 2 (versican)*	Hs.81800	BF590263	1679.69	5982.22	0.280780379	4.7E-07
213905_x_at	biglycan	Hs.821	AA845258	5025.39	17320.39	0.290143005	6.45E-10
203362_s_at	MAD2 mitotic arrest deficient-like 1 (yeast)	Hs.79078	NM_002358.2	1126.73	3794.7	0.296922023	4.29E-07
209596_at	adlican	Hs.72157	AF245505.1	9872.98	31833.51	0.310144247	9.57E-06
217762_s_at	RAB31, member RAS oncogene family	Hs.223025	BE789881	6239.5	20080.05	0.310731298	8.96E-07
212353_at	sulfatase FP	Hs.70823	AW043713	3298.13	10610.47	0.310837314	2.29E-07
221729_at	collagen, type V, alpha 2	Hs.82985	NM_000393.1	8089.9	25965.7	0.311561021	1.79E-08
202503_s_at	KIAA0101 gene product	Hs.81892	NM_014736.1	4140.8	13277.67	0.311861946	8.17E-09
200660_at	S100 calcium binding protein A11 (calgizzarin)	Hs.256290	NM_005620.1	19359.81	60412.84	0.320458532	1.37E-08
210046_s_at	isocitrate dehydrogenase 2 (NADP+), mitochondrial	Hs.5337	U52144.1	6598.83	20503.1	0.321845477	2.19E-06
218039_at	nucleolar protein ANKT	Hs.279905	NM_016359.1	2649.43	8088.17	0.327568535	4.71E-08
200838_at	cathepsin B	Hs.297939	NM_001908.1	8903.1	26015.64	0.342221064	5.79E-09
208850_s_at	Thy-1 cell surface antigen	Hs.125359	AL558479	3334.94	9742.28	0.342316172	1.02E-07
215438_x_at	G1 to S phase transition 1	Hs.2707	BE906054	3749.34	10880.78	0.344583752	2.4E-07
213274_s_at	cathepsin B	Hs.297939	BE875786	5290.88	15121.92	0.349881497	9.49E-10
214352_s_at	v-Ki-ras2 Kirsten rat sarcoma 2 viral oncogene homolog	Hs.351221	BF673699	8905.97	25327.68	0.351629916	4.28E-13
208691_at	transferrin receptor (p90, CD71)	Hs.77356	BC001188.1	10599.34	30095.24	0.352193237	1.63E-06
211161_s_at	collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant)	Hs.119571	AF130082.1	16874.98	47522.98	0.355090948	4.8E-07
200887_s_at	signal transducer and activator of transcription 1, 91kD	Hs.21486	NM_007315.1	11865.1	33057.82	0.358919614	2.31E-07
222077_s_at	Rac GTPase activating protein 1	Hs.23900	AU153848	2198.49	6100.35	0.360387519	1.65E-08
212057_at	KIAA0182 protein	Hs.75909	D80004.1	5085.42	14109.59	0.360422946	9.01E-06
222039_at	hypothetical protein FLJ11029	Hs.274448	AA292789	985.61	2733.2	0.360606615	6.79E-06
202391_at	brain abundant, membrane attached signal protein 1	Hs.79516	NM_006317.1	6613.73	18202.02	0.36335143	1.85E-06
222158_s_at	CGI-146 protein	Hs.42409	AF229834.1	2670.29	7278.07	0.366895345	1.63E-06
214435_x_at	v-ral simian leukemia viral oncogene homolog A (ras related)	Hs.288757	NM_005402.1	1882.24	5097.71	0.369232459	2.9E-09
208998_at	uncoupling protein 2 (mitochondrial, proton carrier)	Hs.80658	U94592.1	10979.98	29619.79	0.370697429	2.5E-08
205436_s_at	H2A histone family, member X	Hs.147097	NM_002105.1	4050.78	10910.21	0.371283413	2.31E-08
209218_at	squalene epoxidase	Hs.71465	AF098865.1	4862.95	12883.73	0.377448922	2.68E-06
219148_at	T-LAK cell-originated protein kinase	Hs.104741	NM_018492.1	783.67	2061.19	0.380202698	1.27E-05

214710_s_at	cyclin B1	Hs.23960	BE407516	1750.12	4576.64	0.382402811	1.41E-06
202736_s_at	U6 snRNA-associated Sm-like protein	Hs.76719	NM_012321.1	3258.86	8432.11	0.38648215	7.8E-07
201954_at	actin related protein 2/3 complex, subunit 1B (41 kD)	Hs.11538	NM_005720.1	5792.32	14857.02	0.389870916	1.98E-09
AFFX-HUMISGF3A/M97935_3_at	signal transducer and activator of transcription 1, 91kD	Hs.21486	M97935	8912.27	22688.41	0.392811572	7.83E-08
202954_at	ubiquitin-conjugating enzyme E2C	Hs.93002	NM_007019.1	3982.35	10133.97	0.392970376	1.13E-06
209945_s_at	glycogen synthase kinase 3 beta	Hs.78802	BC000251.1	2414.33	6121.16	0.394423606	4.26E-08
213553_x_at	apolipoprotein C-I	Hs.268571	W79394	6342.73	15981.27	0.396885229	6.13E-06
210004_at	oxidised low density lipoprotein (lectin-like) receptor 1	Hs.77729	AF035776.1	929.49	2322.52	0.400207533	9.33E-06
208091_s_at	hypothetical protein DKFZp564K0822	Hs.4750	NM_030796.1	7908.33	19735.4	0.400717999	4.32E-09

Upregulated in normals

Gene Name	Gene Description	UniGene	GeneBank	Normal_median	Ttumor _median	Fold change (norr	P-value
202037_s_at	secreted frizzled-related protein 1	Hs.7306	NM_003012.2	59365.66	5359.35	11.07702613	7.16E-11
212730_at	KIAA0353 protein	Hs.10587	AK026420.1	46331.26	4401.76	10.52562157	1.72E-12
205051_s_at	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	Hs.81665	NM_000222.1	30870.31	3453.96	8.937657066	1.28E-11
203881_s_at	dystrophin (muscular dystrophy, Duchenne and Becker types)	Hs.169470	NM_004010.1	9702.27	1267.79	7.652899928	5.88E-17
209292_at	inhibitor of DNA binding 4, dominant negative helix-loop-helix protein	Hs.34853	NM_001546.1	6037.09	864.39	6.984220086	8.13E-11
209291_at	inhibitor of DNA binding 4, dominant negative helix-loop-helix protein	Hs.34853	NM_001546.1	19487.35	2908.02	6.701243458	7.26E-09
202035_s_at	secreted frizzled-related protein 1	Hs.7306	AI332407	8226.47	1233.99	6.666561317	1.2E-05
206825_at	oxytocin receptor	Hs.2820	NM_000916.2	14315.07	2188.79	6.540175165	2.48E-15
218706_s_at	hypothetical protein FLJ21313	Hs.235445	AW575493	15578.77	2719.59	5.728352435	1.21E-13
202350_s_at	matrilin 2	Hs.19368	NM_002380.2	11301.25	2099.9	5.381803895	2.25E-07
211737_x_at	pleiotrophin (heparin binding growth factor 8, neurite growth-promoting factor 1)	Hs.44	BC005916.1	19118.74	3681.29	5.193489239	1.98E-09
209863_s_at	tumor protein p63	Hs.137569	AF091627.1	15557.74	3073.13	5.062506305	5.23E-12
218087_s_at	SH3-domain protein 5 (ponsin)	Hs.108924	NM_015385.1	7983.63	1692.15	4.718039181	1.17E-12
219795_at	solute carrier family 6 (neurotransmitter transporter), member 14	Hs.162211	NM_007231.1	3443.96	767.46	4.487478175	3.52E-06
202342_s_at	tripartite motif-containing 2	Hs.12372	NM_015271.1	8892.84	2088.2	4.258615075	5.46E-07
209290_s_at	nuclear factor I/B	Hs.33287	BC001283.1	51664.48	12407.42	4.16399864	3.45E-06
213029_at	Homo sapiens mRNA; cDNA DKFZp564H1916 (from clone DKFZp564H1916)	Hs.326416	AL110126.1	31908.67	7680.26	4.154634088	1.19E-10
203706_s_at	frizzled homolog 7 (Drosophila)	Hs.173859	NM_003507.1	19052.38	4610.75	4.132165049	3.3E-07
209392_at	ectonucleotide pyrophosphatase/phosphodiesterase 2 (autotaxin)	Hs.174185	L35594.1	12733.37	3091.99	4.118179554	9.92E-10
214598_at	claudin 8	Hs.162209	AL049977.1	8208.2	1993.78	4.11690357	7.3E-07
203065_s_at	caveolin 1, caveolae protein, 22kD	Hs.74034	NM_001753.2	15611.14	3827.36	4.078827181	1.67E-12
204731_at	transforming growth factor, beta receptor III (betaglycan, 300kD)	Hs.342874	NM_003243.1	12204.26	3072.8	3.971706587	5.14E-06
218330_s_at	retinoic acid inducible in neuroblastoma	Hs.23467	NM_018162.1	12668.28	3289.49	3.851138018	2.24E-08
203323_at	caveolin 2	Hs.139851	BF197655	11789.6	3069.88	3.8404107	1E-15
218804_at	hypothetical protein FLJ10261	Hs.26176	NM_018043.1	12822.63	3377.19	3.796834054	1.74E-06
206481_s_at	LIM domain binding 2	Hs.4980	NM_001290.1	7116.81	1895.62	3.754344225	1.03E-09
208370_s_at	Down syndrome critical region gene 1	Hs.184222	NM_004414.2	21019.72	5602.52	3.751833104	7.5E-07
211726_s_at	flavin containing monooxygenase 2	Hs.132821	BC005894.1	17812.59	4796.43	3.713718328	3.49E-08

201012_at	annexin A1	Hs.78225	NM_000700.1	41241.85	11106.89	3.713177136	3.91E-10
212097_at	caveolin 1, caveolae protein, 22kD	Hs.74034	AU147399	23596.76	6367.19	3.705992753	3.08E-15
209170_s_at	glycoprotein M6B	Hs.5422	AF016004.1	8790.1	2373.92	3.702778527	2.01E-07
209160_at	aldo-keto reductase family 1, member C3 (3-alpha hydroxysteroid dehydrogenase, type II)	Hs.78183	AB018580.1	6068.7	1643.09	3.693467795	2.12E-07
202746_at	integral membrane protein 2A	Hs.17109	AL021786	14250.79	3939.27	3.617622047	2.69E-10
209894_at	leptin receptor	Hs.226627	U50748.1	3660.94	1016.43	3.601763033	5.5E-11
203324_s_at	caveolin 2	Hs.139851	NM_001233.1	6068.91	1715.26	3.538186631	2.97E-10
204719_at	ATP-binding cassette, sub-family A (ABC1), member 8	Hs.38095	NM_007168.1	4833.57	1388.04	3.482298781	5.56E-08
203549_s_at	lipoprotein lipase	Hs.180878	NM_000237.1	10789.01	3131.46	3.44536095	9.05E-11
206115_at	early growth response 3	Hs.74088	NM_004430.1	12017.1	3516.09	3.41774528	5.81E-06
219935_at	a disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 5 (aggrecanase-2)	Hs.58324	NM_007038.1	9376.24	2753.5	3.405207917	3.35E-12
201656_at	integrin, alpha 6	Hs.227730	NM_000210.1	9626.26	2893.95	3.326339432	4.04E-07
205463_s_at	platelet-derived growth factor alpha polypeptide	Hs.37040	NM_002607.1	8648.24	2619.44	3.301560639	3.12E-12
823_at	small inducible cytokine subfamily D (Cys-X3-Cys), member 1 (fractalkine, neurotactin)	Hs.80420	U84487	12990.21	3946.33	3.291719142	8.6E-07
213032_at	Homo sapiens mRNA; cDNA DKFZp564H1916 (from clone DKFZp564H1916)	Hs.326416	AL110126.1	12729.9	3880.97	3.280082041	8.56E-06
217047_s_at	KIAA0914 gene product	Hs.177664	AK027138.1	9278.12	2871.79	3.230779409	5.28E-09
209465_x_at	pleiotrophin (heparin binding growth factor 8, neurite growth-promoting factor 1)	Hs.44	AL565812	7512.2	2334.46	3.217960471	7.53E-08
207808_s_at	protein S (alpha)	Hs.64016	NM_000313.1	5027.75	1573.15	3.195976226	1.7E-09
209289_at	nuclear factor I/B	Hs.33287	AI700518	43037.8	13478.56	3.193056232	3.62E-06
209185_s_at	insulin receptor substrate 2	Hs.143648	AF073310.1	19990.69	6334.2	3.155992864	1.39E-06
202552_s_at	cysteine-rich motor neuron 1	Hs.19280	NM_016441.1	8386.55	2721.46	3.081636328	8.31E-09
203688_at	polycystic kidney disease 2 (autosomal dominant)	Hs.82001	NM_000297.1	7543.97	2462.41	3.063653088	3.73E-10
222162_s_at	a disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 1	Hs.8230	AK023795.1	10496.22	3485.94	3.01101568	3.81E-06
211685_s_at	neurocalcin delta	Hs.90063	AF251061.1	9352.32	3133.91	2.984233753	1.78E-08
213900_at	Friedreich ataxia region gene X123	Hs.77889	AA524029	11954.68	4037.3	2.961058133	1.26E-11
222372_at	ESTs, Weakly similar to ALU1_HUMAN ALU SUBFAMILY J SEQUENCE CONTAMINATION WARNING ENTRY [H.sapiens]	Hs.291289	AW971248	8049.26	2718.48	2.960941408	4.62E-06
201540_at	four and a half LIM domains 1	Hs.239069	NM_001449.1	17627.89	6015.25	2.930533228	4.28E-08
212254_s_at	bullous pemphigoid antigen 1 (230/240kD)	Hs.198689	BG253119	19972.78	6991.03	2.856915219	1.32E-09
213353_at	ATP-binding cassette, sub-family A (ABC1), member 5	Hs.180513	BF693921	5730.62	2019.34	2.837867818	3.71E-10
205498_at	growth hormone receptor	Hs.125180	NM_000163.1	7384.79	2603.42	2.836572662	4.63E-06
215016_x_at	bullous pemphigoid antigen 1 (230/240kD)	Hs.198689	BC004912.1	19089.82	6747.39	2.829215445	3.72E-09
208944_at	transforming growth factor, beta receptor II (70-80kD)	Hs.82028	D50683.1	18938.86	6698.52	2.827320065	7.59E-12
210839_s_at	ectonucleotide pyrophosphatase/phosphodiesterase 2 (autotaxin)	Hs.174185	D45421.1	7024.74	2493.07	2.817706683	4.26E-13
218901_at	phospholipid scramblase 4	Hs.182538	NM_020353.1	8923.62	3169.64	2.815341805	1.56E-10
209466_x_at	pleiotrophin (heparin binding growth factor 8, neurite growth-promoting factor 1)	Hs.44	M57399.1	18099.82	6464.73	2.799779728	4.27E-08
200795_at	SPARC-like 1 (mast9, hevin)	Hs.75445	NM_004684.1	62309.15	22325.59	2.790929601	4.78E-07
202973_x_at	KIAA0914 gene product	Hs.177664	NM_014883.1	11301.89	4053.46	2.788208099	4.1E-07
218723_s_at	RGC32 protein	Hs.76640	NM_014059.1	13133.05	4722.25	2.781100111	2.13E-07
213375_s_at	hypothetical gene CG018	Hs.22174	N80918	9894.2	3571.88	2.770025869	2.77E-09

221841_s_at	Kruppel-like factor 4 (gut)	Hs.356370	BF514079	17464.66	6347.92	2.751241351	1.3E-06
218276_s_at	WW45 protein	Hs.288906	NM_021818.1	6994.97	2552.32	2.740632052	4.14E-09
212463_at	Homo sapiens mRNA; cDNA DKFZp564J0323 (from clone DKFZp564J0323)	Hs.99766	BE379006	23386.73	8711.13	2.684695327	2.02E-08
213486_at	hypothetical protein DKFZp761N09121	Hs.6421	BF435376	4412.93	1649.6	2.675151552	2.78E-14
206306_at	ryanodine receptor 3	Hs.9349	NM_001036.1	2449.43	926.73	2.643089141	3.38E-09
212675_s_at	KIAA0582 protein	Hs.79507	AB011154.1	6645.48	2532.1	2.624493503	4.88E-12
200762_at	dihydropyrimidinase-like 2	Hs.173381	NM_001386.1	24509.97	9355.96	2.619717271	1.4E-08
207480_s_at	Meis1, myeloid ecotropic viral integration site 1 homolog 2 (mouse)	Hs.104105	NM_020149.1	5180.76	2010.23	2.577197634	2.37E-07
219091_s_at	EMILIN-like protein EndoGlyx-1	Hs.127216	NM_024756.1	6277.33	2442.04	2.5705271	4.58E-13
219304_s_at	spinal cord-derived growth factor-B	Hs.112885	NM_025208.1	10905.82	4319.06	2.525044801	9.33E-10
207542_s_at	aquaporin 1 (channel-forming integral protein, 28kD)	Hs.74602	NM_000385.2	8557.32	3405.56	2.512749739	8.69E-07
211998_at	H3 histone, family 3B (H3.3B)	Hs.180877	NM_005324.1	10030.86	3995.83	2.510332021	8.65E-06
204115_at	guanine nucleotide binding protein 11	Hs.83381	NM_004126.1	5852.14	2337.15	2.50396423	2.41E-07
202016_at	mesoderm specific transcript homolog (mouse)	Hs.79284	NM_002402.1	21998.29	8805.67	2.498196049	1.05E-07

Probe = Affymetrix Probe Sequence
Description = Gene name and annotation
Unigene = Unigene Number (NCBI)
Genbank = Genbank Accession Number

Median = Median expression value in Normals or Tumors
Fold change = Ratio of expression values (normals/tumors)
P-value = t-test significance

Table 4b : Minimal Geneset for the Classification of Normal vs Tumor

Upregulated in Tumors

Probe	Gene Description	UniGene	GeneBank
201954_at	actin related protein 2/3 complex, subunit 1B (41 kD)	Hs.11538	NM_005720.1
213905_x_at	biglycan	Hs.821	AA845258
201261_x_at	biglycan	Hs.821	BC002416.1
202391_at	brain abundant, membrane attached signal protein 1	Hs.79516	NM_006317.1
205483_s_at	interferon-stimulated protein, 15 kDa	Hs.833	NM_005101.1
221729_at	collagen, type V, alpha 2	Hs.82985	NM_000393.1
211161_s_at	collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant)	Hs.119571	AF130082.1
201422_at	interferon, gamma-inducible protein 30	Hs.14623	NM_006332.1
203936_s_at	matrix metalloproteinase 9 (gelatinase B, 92kD gelatinase, 92kD type IV collagenase)	Hs.151738	NM_004994.1
210004_at	oxidised low density lipoprotein (lectin-like) receptor 1	Hs.77729	AF035776.1
208998_at	uncoupling protein 2 (mitochondrial, proton carrier)	Hs.80658	U94592.1
222039_at	hypothetical protein FLJ11029	Hs.274448	AA292789

Upregulated in Normals

Probe	Gene Description	UniGene	GeneBank
209160_at	aldo-keto reductase family 1, member C3 (3-alpha hydroxysteroid dehydrogenase, type II)	Hs.78183	AB018580.1
201012_at	annexin A1	Hs.78225	NM_000700.1
204719_at	ATP-binding cassette, sub-family A (ABC1), member 8	Hs.38095	NM_007168.1
221841_s_at	Kruppel-like factor 4 (gut)	Hs.356370	BF514079
210839_s_at	ectonucleotide pyrophosphatase/phosphodiesterase 2 (autotaxin)	Hs.174185	D45421.1
209392_at	ectonucleotide pyrophosphatase/phosphodiesterase 2 (autotaxin)	Hs.174185	L35594.1
201540_at	four and a half LIM domains 1	Hs.239069	NM_001449.1
202342_s_at	tripartite motif-containing 2	Hs.12372	NM_015271.1
209185_s_at	insulin receptor substrate 2	Hs.143648	AF073310.1
209894_at	leptin receptor	Hs.226627	U50748.1
206481_s_at	LIM domain binding 2	Hs.4980	NM_001290.1
202016_at	mesoderm specific transcript homolog (mouse)	Hs.79284	NM_002402.1
209290_s_at	nuclear factor I/B	Hs.33287	BC001283.1
218901_at	phospholipid scramblase 4	Hs.182538	NM_020353.1
209466_x_at	pleiotrophin (heparin binding growth factor 8, neurite growth-promoting factor 1)	Hs.44	M57399.1
211737_x_at	pleiotrophin (heparin binding growth factor 8, neurite growth-promoting factor 1)	Hs.44	BC005916.1
202037_s_at	secreted frizzled-related protein 1	Hs.7306	NM_003012.2
205051_s_at	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	Hs.81665	NM_000222.1
212730_at	KIAA0353 protein	Hs.10587	AK026420.1
218330_s_at	retinoic acid inducible in neuroblastoma	Hs.23467	NM_018162.1

Table 5A : CGS for ER and ERBB2 Classification

ER Classification Genes

Probe	Gene Name	Unigene	GenBank	Regulation
205225_at	estrogen receptor 1	Hs.1657	NM_000125.1	+
203963_at	carbonic anhydrase XII	Hs.5338	NM_001218.2	+
209602_s_at	GATA binding protein 3	Hs.169946	AI796169	+
214164_x_at	adaptor-related protein complex 1, gamma 1 subunit	Hs.5344	BF752277	+
202089_s_at	LIV-1 protein, estrogen regulated	Hs.79136	NM_012319.2	+
212956_at	KIAA0882 protein	Hs.90419	AB020689.1	+
214440_at	N-acetyltransferase 1 (arylamine N-acetyltransferase)	Hs.155956	NM_000662.1	+
206754_s_at	cytochrome P450, subfamily IIB (phenobarbital-inducible), polypeptide 6	Hs.1360	NM_000767.2	+
222212_s_at	LAG1 longevity assurance homolog 2 (S. cerevisiae)	Hs.285976	AK001105.1	+
218195_at	hypothetical protein FLJ12910	Hs.15929	NM_024573.1	+
205862_at	KIAA0575 gene product	Hs.193914	NM_014668.1	+
212195_at	Homo sapiens mRNA; cDNA DKFZp564F053 (from clone DKFZp564F053)	Hs.71968	AL049265.1	+
208682_s_at	melanoma antigen, family D, 2	Hs.4943	AF126181.1	+
202342_s_at	tripartite motif-containing 2	Hs.12372	NM_015271.1	-
209459_s_at	NPD009 protein	Hs.283675	AF237813.1	+
201037_at	phosphofructokinase, platelet	Hs.99910	NM_002627.1	-
203571_s_at	adipose specific 2	Hs.74120	NM_006829.1	+
214088_s_at	fucosyltransferase 3 (galactoside 3(4)-L-fucosyltransferase, Lewis blood group included)	Hs.169238	AW080549	-
201976_s_at	myosin X	Hs.61638	NM_012334.1	-
218502_s_at	trichorhinophalangeal syndrome I	Hs.26102	NM_014112.1	+
203221_at	transducin-like enhancer of split 1 (E(sp1) homolog, Drosophila)	Hs.28935	AI951720	-
207002_s_at	pleiomorphic adenoma gene-like 1	Hs.75825	NM_002656.1	-
207030_s_at	cysteine and glycine-rich protein 2	Hs.10526	NM_001321.1	-
204623_at	trefoil factor 3 (intestinal)	Hs.352107	NM_003226.1	+
205009_at	trefoil factor 1 (breast cancer, estrogen-inducible sequence expressed in)	Hs.350470	NM_003225.1	+

Regulation = On (+) or Off (-) in an ER+ tumor

Table 5B:ERBB2 Classification Genes

Probe	Gene Name	ˈUnigene	GenBank	Regulation
216836_s_at	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)	Hs.323910	X03363.1	+
210761_s_at	growth factor receptor-bound protein 7	Hs.86859	AB008790.1	+
202991_at	steroidogenic acute regulatory protein related	Hs.77628	NM_006804.1	+
55616_at	hypothetical gene MGC9753	Hs.91668	AI703342	+
214203_s_at	proline dehydrogenase (oxidase) 1	Hs.343874	AA074145	+
213557_at	KIAA0904 protein	Hs.278346	AW305119	+
220149_at	hypothetical protein FLJ22671	Hs.193745	NM_024861.1	+
215659_at	Homo sapiens cDNA: FLJ21521 fis, clone COL05880	Hs.306777	AK025174.1	+
219233_s_at	hypothetical protein PRO2521	Hs.19054	NM_018530.1	+
203497_at	PPAR binding protein	Hs.15589	NM_004774.1	+
219226_at	CDC2-related protein kinase 7	Hs.123073	NM_016507.1	+
202712_s_at	creatine kinase, mitochondrial 1 (ubiquitous)	Hs.153998	NM_020990.2	+
204285_s_at	phorbol-12-myristate-13-acetate-induced protein 1	Hs.96	AI857639	-
205225_at	estrogen receptor 1	Hs.1657	NM_000125.1	-
214614_at	homeo box HB9	Hs.37035	AI738662	+
202917_s_at	S100 calcium binding protein A8 (calgranulin A)	Hs.100000	NM_002964.2	+
219429_at	fatty acid hydroxylase	Hs.249163	NM_024306.1	+
208614_s_at	filamin B, beta (actin binding protein 278)	Hs.81008	M62994.1	-
204029_at	cadherin, EGF LAG seven-pass G-type receptor 2 (flamingo homolog, Drosophila)	Hs.57652	NM_001408.1	-
216401_x_at	Homo sapiens partial IGKV gene for immunoglobulin kappa chain variable region, clone 38	Hs.307136	AJ408433	+
203685_at	B-cell CLL/lymphoma 2	Hs.79241	NM_000633.1	-
216576_x_at	Homo sapiens isolate donor N clone N88K immunoglobulin kappa light chain variable region mRNA, partial cds	Hs.247910	AF103529.1	+
211138_s_at	kynurenine 3-monooxygenase (kynurenine 3-hydroxylase)	Hs.107318	BC005297.1	+
202039_at	TGFB1-induced anti-apoptotic factor 1	Hs.75822	NM_004740.1	+
203627_at	insulin-like growth factor 1 receptor	Hs.239176	NM_000875.2	-
204863_s_at	interleukin 6 signal transducer (gp130, oncostatin M receptor)	Hs.82065	BE856546	-

Table 6a : Predictor Sets for Molecular Subtype Using OVA SVM

Luminal A

Probe	Gene Description	UniGene	GeneBank
201030_x_at	lactate dehydrogenase B	Hs.234489	NM_002300.1
201525_at	apolipoprotein D	Hs.75736	NM_001647.1
201688_s_at	tumor protein D52	Hs.2384	BE974098
201754_at	cytochrome c oxidase subunit VIc	Hs.351875	NM_004374.1
202376_at	serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3	Hs.234726	NM_001085.2
202555_s_at	myosin, light polypeptide kinase	Hs.211582	NM_005965.1
202746_at	integral membrane protein 2A	Hs.17109	AL021786
202991_at	steroidogenic acute regulatory protein related	Hs.77628	NM_006804.1
203627_at	insulin-like growth factor 1 receptor	Hs.239176	NM_000875.2
203749_s_at	retinoic acid receptor, alpha	Hs.250505	AI806984
204198_s_at	runt-related transcription factor 3	Hs.170019	AA541630
204304_s_at	prominin-like 1 (mouse)	Hs.112360	NM_006017.1
205225_at	estrogen receptor 1	Hs.1657	NM_000125.1
205471_s_at	dachshund homolog (Drosophila)	Hs.63931	AW772082
206378_at	secretoglobin, family 2A, member 2	Hs.46452	NM_002411.1
208711_s_at	cyclin D1 (PRAD1: parathyroid adenomatosis 1)	Hs.82932	BC000076.1
209016_s_at	keratin 7	Hs.23881	BC002700.1
209290_s_at	nuclear factor I/B	Hs.33287	BC001283.1
209292_at	inhibitor of DNA binding 4, dominant negative helix-loop-helix protein	Hs.34853	NM_001546.1
209351_at	keratin 14 (epidermolysis bullosa simplex, Dowling-Meara, Koebner)	Hs.117729	BC002690.1
209396_s_at	chitinase 3-like 1 (cartilage glycoprotein-39)	Hs.75184	M80927.1
209465_x_at	pleiotrophin (heparin binding growth factor 8, neurite growth-promoting factor 1)	Hs.44	AL565812
209863_s_at	tumor protein p63	Hs.137569	AF091627.1
211538_s_at	heat shock 70kD protein 2	Hs.75452	U56725.1
211726_s_at	flavin containing monooxygenase 2	Hs.132821	BC005894.1
211737_x_at	pleiotrophin (heparin binding growth factor 8, neurite growth-promoting factor 1)	Hs.44	BC005916.1
211958_at	Homo sapiens, clone IMAGE:4183312, mRNA, partial cds	Hs.180324	L27560.1
211959_at	Homo sapiens, clone IMAGE:4183312, mRNA, partial cds	Hs.180324	L27560.1
212730_at	KIAA0353 protein	Hs.10587	AK026420.1
213564_x_at	lactate dehydrogenase B	Hs.234489	BE042354
216836_s_at	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)	Hs.323910	X03363.1
217762_s_at	RAB31, member RAS oncogene family	Hs.223025	BE789881
217838_s_at	RNB6	Hs.241471	NM_016337.1
218532_s_at	hypothetical protein FLJ20152	Hs.82273	NM_019000.1
221765_at	Homo sapiens mRNA full length insert cDNA clone EUROIMAGE 1287006	Hs.23703	BF970427

ER- Subtype II

Probe	Gene Description	UniGene	GeneBank
200099_s_at	Human DNA sequence from clone RP11-486O22 on chromosome 10 Contains the 3part of a gene for KIAA1128 protein, a novel pseudogene, a gene for protein similar to RPS3A (ribosomal protein S3A), ESTs, STSs, GSSs and CpG islands	Hs.307132	AL356115
37892_at	collagen, type XI, alpha 1	Hs.82772	J04177
39248_at	aquaporin 3	Hs.234642	N74607
200606_at	desmoplakin (DPI, DPII)	Hs.349499	NM_004415.1
200706_s_at	LPS-induced TNF-alpha factor	Hs.76507	NM_004862.1
200749_at	RAN, member RAS oncogene family	Hs.10842	BF112006
200811_at	cold inducible RNA binding protein	Hs.119475	NM_001280.1
200823_x_at	ribosomal protein L29	Hs.350068	NM_000992.1
200853_at	H2A histone family, member Z	Hs.119192	NM_002106.1
200925_at	cytochrome c oxidase subunit VIa polypeptide 1	Hs.180714	NM_004373.1
200935_at	calreticulin	Hs.16488	NM_004343.2
201054_at	heterogeneous nuclear ribonucleoprotein A0	Hs.77492	BE966599
201080_at	phosphatidylinositol-4-phosphate 5-kinase, type II, beta	Hs.6335	BF338509
201131_s_at	cadherin 1, type 1, E-cadherin (epithelial)	Hs.194657	NM_004360.1
201134_x_at	cytochrome c oxidase subunit VIIc	Hs.3462	NM_001867.1
201291_s_at	topoisomerase (DNA) II alpha (170kD)	Hs.156346	NM_001067.1
201349_at	solute carrier family 9 (sodium/hydrogen exchanger), isoform 3 regulatory factor 1	Hs.184276	NM_004252.1
201431_s_at	dihydropyrimidinase-like 3	Hs.74566	NM_001387.1
201552_at	lysosomal-associated membrane protein 1	Hs.150101	NM_005561.2
201688_s_at	tumor protein D52	Hs.2384	BE974098
201689_s_at	tumor protein D52	Hs.2384	BE974098
201830_s_at	neuroepithelial cell transforming gene 1	Hs.25155	NM_005863.1
201890_at	ribonucleotide reductase M2 polypeptide	Hs.75319	NM_001034.1
201892_s_at	IMP (inosine monophosphate) dehydrogenase 2	Hs.75432	NM_000884.1
201903_at	ubiquinol-cytochrome c reductase core protein I	Hs.119251	NM_003365.1
201925_s_at	decay accelerating factor for complement (CD55, Cromer blood group system)	Hs.1369	NM_000574.1
201946_s_at	chaperonin containing TCP1, subunit 2 (beta)	Hs.6456	AL545982
202071_at	syndecan 4 (amphiglycan, ryudocan)	Hs.252189	NM_002999.1
202088_at	LIV-1 protein, estrogen regulated	Hs.79136	AI635449
202291_s_at	matrix Gla protein	Hs.365706	NM_000900.1
202376_at	serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3	Hs.234726	NM_001085.2
202489_s_at	FXYD domain-containing ion transport regulator 3	Hs.301350	BC005238.1

202704_at	transducer of ERBB2, 1	Hs.178137	AA675892
203202_at	HIV-1 rev binding protein 2	Hs.154762	AI950314
203627_at	insulin-like growth factor 1 receptor	Hs.239176	NM_000875.2
203628_at	insulin-like growth factor 1 receptor	Hs.239176	NM_000875.2
203789_s_at	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3C	Hs.171921	NM_006379.1
203892_at	WAP four-disulfide core domain 2	Hs.2719	NM_006103.1
203915_at	monokine induced by gamma interferon	Hs.77367	NM_002416.1
203929_s_at	Homo sapiens cDNA FLJ31424 fis, clone NT2NE2000392	Hs.101174	NM_016835.1
203963_at	carbonic anhydrase XII	Hs.5338	NM_001218.2
204018_x_at	hemoglobin, alpha 1	Hs.272572	NM_000558.2
204031_s_at	poly(rC) binding protein 2	Hs.63525	NM_005016.1
204320_at	collagen, type XI, alpha 1	Hs.82772	NM_001854.1
204457_s_at	growth arrest-specific 1	Hs.65029	NM_002048.1
205225_at	estrogen receptor 1	Hs.1657	NM_000125.1
205428_s_at	calbindin 2, (29kD, calretinin)	Hs.106857	NM_001740.2
205453_at	homeo box B2	Hs.2733	NM_002145.1
205887_x_at	mutS homolog 3 (E. coli)	Hs.42674	NM_002439.1
205941_s_at	collagen, type X, alpha 1(Schmid metaphyseal chondrodysplasia)	Hs.179729	AI376003
206211_at	selectin E (endothelial adhesion molecule 1)	Hs.89546	NM_000450.1
206916_x_at	tyrosine aminotransferase	Hs.161640	NM_000353.1
207721_x_at	histidine triad nucleotide binding protein 1	Hs.256697	NM_005340.1
208702_x_at	amyloid beta (A4) precursor-like protein 2	Hs.279518	BC000373.1
208703_s_at	amyloid beta (A4) precursor-like protein 2	Hs.279518	BC000373.1
208711_s_at	cyclin D1 (PRAD1: parathyroid adenomatosis 1)	Hs.82932	BC000076.1
208764_s_at	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit c (subunit 9), isoform 2	Hs.89399	D13119.1
208791_at	clusterin (complement lysis inhibitor, SP-40,40, sulfated glycoprotein 2, testosterone-repressed prostate message 2, apolipoprotein J)	Hs.75106	M25915.1
208792_s_at	clusterin (complement lysis inhibitor, SP-40,40, sulfated glycoprotein 2, testosterone-repressed prostate message 2, apolipoprotein J)	Hs.75106	M25915.1
208826_x_at	histidine triad nucleotide binding protein 1	Hs.256697	U27143.1
208950_s_at	aldehyde dehydrogenase 7 family, member A1	Hs.74294	BC002515.1
209035_at	midkine (neurite growth-promoting factor 2)	Hs.82045	M69148.1
209069_s_at	H3 histone, family 3B (H3.3B)	Hs.180877	BC001124.1
209112_at	cyclin-dependent kinase inhibitor 1B (p27, Kip1)	Hs.238990	BC001971.1
209116_x_at	hemoglobin, beta	Hs.155376	M25079.1
209143_s_at	chloride channel, nucleotide-sensitive, 1A	Hs.84974	AF005422.1

209351_at	keratin 14 (epidermolysis bullosa simplex, Dowling-Meara, Koebner)	Hs.117729	BC002690.1
209369_at	annexin A3	Hs.1378	M63310.1
209403_at	hypothetical protein DKFZp434P2235	Hs.105891	AL136860.1
209602_s_at	GATA binding protein 3	Hs.169946	AI796169
210163_at	small inducible cytokine subfamily B (Cys-X-Cys), member 11	Hs.103982	AF030514.1
210387_at	H2B histone family, member A	Hs.352109	BC001131.1
210511_s_at	inhibin, beta A (activin A, activin AB alpha polypeptide)	Hs.727	M13436.1
210715_s_at	serine protease inhibitor, Kunitz type, 2	Hs.31439	AF027205.1
210764_s_at	cysteine-rich, angiogenic inducer, 61	Hs.8867	AF003114.1
211113_s_at	ATP-binding cassette, sub-family G (WHITE), member 1	Hs.10237	U34919.1
211404_s_at	amyloid beta (A4) precursor-like protein 2	Hs:279518	BC004371.1
211696_x_at	hemoglobin, beta	Hs.155376	AF349114.1
211745_x_at	hemoglobin, alpha 2	Hs.347939	BC005931.1
211935_at	ADP-ribosylation factor-like 6 interacting protein	Hs.75249	D31885.1
212328_at	KIAA1102 protein	Hs.202949	AK027231.1
212492_s_at	KIAA0876 protein	Hs.301011	AW237172
212692_s_at	vesicle trafficking, beach and anchor containing	Hs.62354	W60686
212942_s_at	KIAA1199 protein	Hs.50081	AB033025.1
212956_at	KIAA0882 protein	Hs.90419	AB020689.1
213557_at	KIAA0904 protein	Hs.278346	AW305119
213764_s_at	Microfibril-associated glycoprotein-2	Hs.300946	AW665892
213765_at	Microfibril-associated glycoprotein-2	Hs.300946	AW665892
214079_at	Homo sapiens cDNA FLJ20338 fis, clone HEP12179	Hs.152677	AK000345.1
214414_x_at	hemoglobin, alpha 2	Hs.347939	T50399
214836_x_at	immunoglobulin kappa constant	Hs.156110	BG536224
215224_at	Homo sapiens cDNA: FLJ21547 fis, clone COL06206	Hs.322680	AK025200.1
215867_x_at	adaptor-related protein complex 1, gamma 1 subunit	Hs.5344	AL050025.1
217014_s_at	Homo sapiens PAC clone RP4-604G5 from 7q22-q31.1	Hs.307354	AC004522
217428_s_at	collagen, type X, alpha 1 (Schmid metaphyseal chondrodysplasia)	Hs.179729	X98568
217704_x_at	ESTs, Moderately similar to ALU7_HUMAN ALU SUBFAMILY SQ SEQUENCE CONTAMINATION WARNING ENTRY [H.sapiens]	Hs.310806	AI820796
217753_s_at	ribosomal protein S26	Hs.299465	NM_001029.1
218237_s_at	solute carrier family 38, member 1	Hs.18272	NM_030674.1
218302_at	uncharacterized hematopoietic stem/progenitor cells protein MDS033	Hs.54960	NM_018468.1
218388_at	6-phosphogluconolactonase	Hs.100071	NM_012088.1
218468_s_at	cysteine knot superfamily 1, BMP antagonist 1	Hs.40098	AF154054.1

218469_at	cysteine knot superfamily 1, BMP antagonist 1	Hs.40098	NM_013372.1
219087_at	asporin (LRR class 1)	Hs.10760	NM_017680.1
219454_at	EGF-like-domain, multiple 6	Hs.12844	NM_015507.2
219734_at	hypothetical protein FLJ20174	Hs.114556	NM_017699.1
219773_at	NADPH oxidase 4	Hs.93847	NM_016931.1
220149_at	hypothetical protein FLJ22671	Hs.193745	NM_024861.1
220864_s_at	cell death-regulatory protein GRIM19	Hs.279574	NM_015965.1
221434_s_at	hypothetical protein DC50	Hs.324521	NM_031210.1
221473_x_at	tumor differentially expressed 1	Hs.272168	U49188.1
221541_at	hypothetical protein DKFZp434B044	Hs.262958	AL136861.1

Basal

Probe	Gene Description	UniGene	GeneBank
202342_s_at	tripartite motif-containing 2	Hs.12372	NM_015271.1
202345_s_at	fatty acid binding protein 5 (psoriasis-associated)	Hs.153179	NM_001444.1
202412_s_at	ubiquitin specific protease 1	Hs.35086	AW499935
203780_at	epithelial V-like antigen 1	Hs.116651	AF275945.1
204580_at	matrix metalloproteinase 12 (macrophage elastase)	Hs.1695	NM_002426.1
205066_s_at	ectonucleotide pyrophosphatase/phosphodiesterase 1	Hs.11951	NM_006208.1
206042_x_at	SNRPN upstream reading frame	Hs.58606	NM_022804.1
206102_at	KIAA0186 gene product	Hs.36232	NM_021067.1
209205_s_at	LIM domain only 4	Hs.3844	BC003600.1
209212_s_at	Kruppel-like factor 5 (intestinal)	Hs.84728	AB030824.1
209351_at	keratin 14 (epidermolysis bullosa simplex, Dowling-Meara, Koebner)	Hs.117729	BC002690.1
212236_x_at	keratin 17	Hs.2785	Z19574
212592_at	Homo sapiens, clone MGC:24130 IMAGE:4692359, mRNA, complete cds	Hs.76325	AV733266
213664_at	solute carrier family 1 (neuronal/epithelial high affinity glutamate transporter, system Xag), member 1	Hs.91139	AW235061
213668_s_at	SRY (sex determining region Y)-box 4	Hs.83484	AI989477
213680_at	keratin 6B	Hs.335952	AI831452
217744_s_at	p53-induced protein PIGPC1	Hs.303125	NM_022121.1
218499_at	Mst3 and SOK1-related kinase	Hs.23643	NM_016542.1
218593_at	hypothetical protein FLJ10377	Hs.274263	NM_018077.1
222039_at	hypothetical protein FLJ11029	Hs.274448	AA292789

ERBB2

Probe	Gene Description	UniGene	GeneBank
55616_at	hypothetical gene MGC9753	Hs.91668	AI703342
201388_at	proteasome (prosome, macropain) 26S subunit, non-ATPase, 3	Hs.9736	NM_002809.1
201525_at	apolipoprotein D	Hs.75736	NM_001647.1
202035_s_at	secreted frizzled-related protein 1	Hs.7306	AI332407
202036_s_at	secreted frizzled-related protein 1	Hs.7306	AF017987.1
202145_at	lymphocyte antigen 6 complex, locus E	Hs.77667	NM_002346.1
202218_s_at	fatty acid desaturase 2	Hs.184641	NM_004265.1
202376_at	serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3	Hs.234726	NM_001085.2
202991_at	steroidogenic acute regulatory protein related	Hs.77628	NM_006804.1
203355_s_at	KIAA0942 protein	Hs.6763	NM_015310.1
203404_at	armadillo repeat protein ALEX2	Hs.48924	NM_014782.1
203439_s_at	stanniocalcin 2	Hs.155223	BC000658.1
203628_at	insulin-like growth factor 1 receptor	Hs.239176	NM_000875.2
203685_at	B-cell CLL/lymphoma 2	Hs.79241	NM_000633.1
204734_at	keratin 15	Hs.80342	NM_002275.1
204942_s_at	aldehyde dehydrogenase 3 family, member B2	Hs.87539	NM_000695.2
205225_at	estrogen receptor 1	Hs.1657	NM_000125.1
205306_x_at	kynurenine 3-monooxygenase (kynurenine 3-hydroxylase)	Hs.107318	AI074145
206165_s_at	chloride channel, calcium activated, family member 2	Hs.241551	NM_006536.2
206378_at	secretoglobin, family 2A, member 2	Hs.46452	NM_002411.1
207076_s_at	argininosuccinate synthetase	Hs.160786	NM_000050.1
207131_x_at	gamma-glutamyltransferase 1	Hs.284380	NM_013430.1
208180_s_at	H4 histone family, member H	Hs.93758	NM_003543.2
208614_s_at	filamin B, beta (actin binding protein 278)	Hs.81008	M62994.1
209016_s_at	keratin 7	Hs.23881	BC002700.1
209603_at	GATA binding protein 3	Hs.169946	AI796169
210163_at	small inducible cytokine subfamily B (Cys-X-Cys), member 11	Hs.103982	AF030514.1
210519_s_at	diaphorase (NADHNADPH) (cytochrome b-5 reductase)	Hs.80706	BC000906.1
210761_s_at	growth factor receptor-bound protein 7	Hs.86859	AB008790.1
211138_s_at	kynurenine 3-monooxygenase (kynurenine 3-hydroxylase)	Hs.107318	BC005297.1
211430_s_at	immunoglobulin heavy constant gamma 3 (G3m marker)	Hs.300697	M87789.1
211641_x_at	gb:L06101.1 /DEF=Human IG VH-region gene, complete cds. /FEA=mRNA /GEN=IGH@ /PROD=immunoglobulin heavy chain V-region /DB_XREF=gi:185526		L06101.1
211645_x_at	gb:M85256.1 /DEF=Homo sapiens immunoglobulin kappa-chain VK-1 (IgK) mRNA, complete cds. /FEA=mRNA /GEN=IgK /PROD=immunoglobulin kappa-chain VK-1 /DB_XREF=gi:186008		M85256.1
211657_at	gb:M18728.1 /DEF=Human nonspecific crossreacting antigen mRNA, complete cds. /FEA=mRNA /GEN=NCA; NCA; NCA /PROD=non-specific cross reacting antigen /DB_XREF=gi:189084		M18728.1
212218_s_at	F-box only protein 9	Hs.11050	NM_012347.1
212281_s_at	hypothetical protein	Hs.199695	L19183.1
214451_at	transcription factor AP-2 beta (activating enhancer binding protein 2 beta)	Hs.33102	NM_003221.1
214669_x_at	Homo sapiens isolate donor N clone N168K immunoglobulin kappa light chain variable region mRNA, partial cds	Hs.306357	BG485135
215176_x_at	immunoglobulin kappa constant	Hs.156110	AW404894
216557_x_at	Homo sapiens mRNA for single-chain antibody, complete cds	Hs.249245	U92706
216836_s_at	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)	Hs.323910	X03363.1
217157_x_at	Homo sapiens isolate donor N clone N8K immunoglobulin kappa light chain variable region mRNA, partial cds	Hs.247911	AF103530.1

217388_s_at	kynureninase (L-kynurenine hydrolase)	Hs.169139	D55639.1
217480_x_at	Human kappa-immunoglobulin germline pseudogene (cos118) variable region (subgroup V kappa I)	Hs.278448	M20812
219768_at	hypothetical protein FLJ22418	Hs.36563	NM_024626.1
220038_at	serum/glucocorticoid regulated kinase-like	Hs.279696	NM_013257.1

Normal/Normal-like

Probe	Gene Description	UniGene	GeneBank
201030_x_at	lactate dehydrogenase B	Hs.234489	NM_002300.1
201792_at	AE binding protein 1	Hs.118397	NM_001129.2
201860_s_at	plasminogen activator, tissue	Hs.274404	NM_000930.1
202037_s_at	secreted frizzled-related protein 1	Hs.7306	NM_003012.2
202218_s_at	fatty acid desaturase 2	Hs.184641	NM_004265.1
202662_s_at	inositol 1,4,5-triphosphate receptor, type 2	Hs.238272	NM_002223.1
202746_at	integral membrane protein 2A	Hs.17109	AL021786
202887_s_at	HIF-1 responsive RTP801	Hs.111244	NM_019058.1
203058_s_at	3'-phosphoadenosine 5'-phosphosulfate synthase 2	Hs.274230	AW299958
203213_at	cell division cycle 2, G1 to S and G2 to M	Hs.334562	AL524035
203325_s_at	collagen, type V, alpha 1	Hs.146428	AI130969
203685_at	B-cell CLL/lymphoma 2	Hs.79241	NM_000633.1
203706_s_at	frizzled homolog 7 (Drosophila)	Hs.173859	NM_003507.1
203755_at	BUB1 budding uninhibited by benzimidazoles 1 homolog beta (yeast)	Hs.36708	NM_001211.2
203789_s_at	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3C	Hs.171921	NM_006379.1
203878_s_at	matrix metalloproteinase 11 (stromelysin 3)	Hs.155324	NM_005940.2
203915_at	monokine induced by gamma interferon	Hs.77367	NM_002416.1
204033_at	thyroid hormone receptor interactor 13	Hs.6566	NM_004237.1
204602_at	dickkopf homolog 1 (Xenopus laevis)	Hs.40499	NM_012242.1
204731_at	transforming growth factor, beta receptor III (betaglycan, 300kD)	Hs.342874	NM_003243.1
205034_at	cyclin E2	Hs.30464	NM_004702.1
205239_at	amphiregulin (schwannoma-derived growth factor)	Hs.270833	NM_001657.1
207714_s_at	serine (or cysteine) proteinase inhibitor, clade H (heat shock protein 47), member 1, (collagen binding protein 1)	Hs.241579	NM_004353.1
208029_s_at	gb:NM_018407.1 /DEF=Homo sapiens putative integral membrane transporter (LC27), mRNA. /FEA=mRNA /GEN=LC27 /PROD=putative integral membrane transporter /DB_XREF=gi:8923827		NM_018407.1
208791_at	clusterin (complement lysis inhibitor, SP-40,40, sulfated glycoprotein 2, testosterone-repressed prostate message 2, apolipoprotein J)	Hs.75106	M25915.1
208792_s_at	clusterin (complement lysis inhibitor, SP-40,40, sulfated glycoprotein 2, testosterone-repressed prostate message 2, apolipoprotein J)	Hs.75106	M25915.1
209071_s_at	regulator of G-protein signalling 5	Hs.24950	AF159570.1
209218_at	squalene epoxidase	Hs.71465	AF098865.1
209291_at	inhibitor of DNA binding 4, dominant negative helix-loop-helix protein	Hs.34853	NM_001546.1
209292_at	inhibitor of DNA binding 4, dominant negative helix-loop-helix protein	Hs.34853	NM_001546.1
209465_x_at	pleiotrophin (heparin binding growth factor 8, neurite growth-promoting factor 1)	Hs.44	AL565812
209687_at	stromal cell-derived factor 1	Hs.237356	U19495.1
210519_s_at	diaphorase (NADHNADPH) (cytochrome b-5 reductase)	Hs.80706	BC000906.1
211657_at	gb:M18728.1 /DEF=Human nonspecific crossreacting antigen mRNA, complete cds. /FEA=mRNA /GEN=NCA; NCA; NCA /PROD=non-specific cross reacting antigen /DB_XREF=gi:189084		M18728.1
211737_x_at	pleiotrophin (heparin binding growth factor 8, neurite growth-promoting factor 1)	Hs.44	BC005916.1
212236_x_at	keratin 17	Hs.2785	Z19574
212254_s_at	bullous pemphigoid antigen 1 (230/240kD)	Hs.198689	BG253119
212592_at	Homo sapiens, clone MGC:24130 IMAGE:4692359, mRNA, complete cds	Hs.76325	AV733266
212730_at	KIAA0353 protein	Hs.10587	AK026420.1
214290_s_at	H2A histone family, member O	Hs.795	AA451996
216836_s_at	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)	Hs.323910	X03363.1
217428_s_at	collagen, type X, alpha 1 (Schmid metaphyseal chondrodysplasia)	Hs.179729	X98568

218087_s_at	SH3-domain protein 5 (ponsin)	Hs.108924	NM_015385.1
219115_s_at	interleukin 20 receptor, alpha	Hs.21814	NM_014432.1
219197_s_at	CEGP1 protein	Hs.222399	AI424243
219215_s_at	solute carrier family 39 (zinc transporter), member 4	Hs.352415	NM_017767.1
219304_s_at	spinal cord-derived growth factor-B	Hs.112885	NM_025208.1
219768_at	hypothetical protein FLJ22418	Hs.36563	NM_024626.1
220038_at	serum/glucocorticoid regulated kinase-like	Hs.279696	NM_013257.1
222155_s_at	hypothetical protein FLJ11856	Hs.6459	AK021918.1

Table 6b : 2 Optimal Predictor Sets Using the GA/MLHD Algorithm

Gene set 1

Probe	Gene	Unigene	GeneBank
200926_at	*ribosomal protein S23*	Hs.3463	NM_001025.1
205225_at	estrogen receptor 1	Hs.1657	NM_000125.1
200670_at	X-box binding protein 1	Hs.149923	NM_005080.1
208248_x_at	amyloid beta (A4) precursor-like protein 2	Hs.279518	NM_001642.1
209343_at	hypothetical protein FLJ13612	Hs.24391	BC002449.1
213399_x_at	ribophorin II	Hs.75722	AI560720
214938_x_at	high-mobility group (nonhistone chromosomal) protein 1	Hs.274472	AF283771.2
207783_x_at	hypothetical protein FLJ20030	Hs.326456	NM_017627.1
204533_at	small inducible cytokine subfamily B (Cys-X-Cys), member 10	Hs.2248	NM_001565.1
204798_at	v-myb myeloblastosis *viral oncogene homolog* (avian)	Hs.1334	NM_005375.1
212790_x_at	ribosomal protein L13a	Hs.119122	BF942308
217276_x_at	serine hydrolase-like	Hs.301947	AL590118.1
213975_s_at	tudor repeat associator with PCTAIRE 2	Hs.283761	AV711904
202428_x_at	diazepam binding inhibitor (GABA receptor modulator, acyl-Coenzyme A binding protein)	Hs.78888	NM_020548.1
200925_at	cytochrome c oxidase subunit VIa polypeptide 1	Hs.180714	NM_004373.1

Gene set 2

Probe	Gene	Unigene	GeneBank
221729_at	collagen, type V, alpha 2	Hs.82985	NM_000393.1
206461_x_at	metallothionein 1H	Hs.2667	NM_005951.1
205509_at	carboxypeptidase B1 (tissue)	Hs.180884	NM_001871.1
212320_at	tubulin, beta polypeptide	Hs.179661	BC001002.1
209043_at	3'-phosphoadenosine 5'-phosphosulfate synthase 1	Hs.3833	AF033026.1
200032_s_at	ribosomal protein L9	Hs.157850	NM_000661.1
202088_at	LIV-1 protein, estrogen regulated	Hs.79136	AI635449
209604_s_at	GATA binding protein 3	Hs.169946	BC003070.1
201892_s_at	IMP (inosine monophosphate) dehydrogenase 2	Hs.75432	NM_000884.1
211896_s_at	decorin	Hs.76152	AF138302.1
201952_at	activated leucocyte cell adhesion molecule	Hs.10247	NM_001627.1
216836_s_at	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)	Hs.323910	X03363.1

TABLE 7

Up Regulated in luminal D

Gene Name	Title	Unigene_Accession	Seq_Derived_From
201422_at	interferon, gamma-inducible protein 30	Hs.14623	NM_006332.1
201577_at	non-metastatic cells 1, protein (NM23A) expressed in	Hs.118638	NM_000269.1
201884_at	carcinoembryonic antigen-related cell adhesion molecule 5	Hs.220529	NM_004363.1
201946_s_at	chaperonin containing TCP1, subunit 2 (beta)	Hs.6456	AL545982
202433_at	UDP-galactose transporter related	Hs.154073	NM_005827.1
202779_s_at	ubiquitin carrier protein	Hs.174070	NM_014501.1
203628_at	insulin-like growth factor 1 receptor	Hs.239176	NM_000875.2
204566_at	protein phosphatase 1D magnesium-dependent, delta isoform	Hs.100980	NM_003620.1
204868_at	immature colon carcinoma transcript 1	Hs.9078	NM_001545.1
211762_s_at	karyopherin alpha 2 (RAG cohort 1, importin alpha 1)	Hs.159557	BC005978.1
211958_at	Homo sapiens, clone IMAGE:4183312, mRNA, partial cds	Hs.180324	L27560.1
211959_at	Homo sapiens, clone IMAGE:4183312, mRNA, partial cds	Hs.180324	L27560.1
217755_at	hematological and neurological expressed 1	Hs.109706	NM_016185.1
218585_s_at	RA-regulated nuclear matrix-associated protein	Hs.126774	NM_016448.1
218732_at	CGI-147 protein	Hs.12677	NM_016077.1
219493_at	hypothetical protein FLJ22009	Hs.123253	NM_024745.1
222039_at	hypothetical protein FLJ11029	Hs.274448	AA292789
222231_s_at	hypothetical protein PRO1855	Hs.283558	AK025328.1

Down Regulated in luminal D

Gene Name	Title	Unigene_Accession [A]	Seq_Derived_From
201667_at	gap junction protein, alpha 1, 43kD (connexin 43)	Hs.74471	NM_000165.2
201939_at	serum-inducible kinase	Hs.3838	NM_006622.1
202291_s_at	matrix Gla protein	Hs.365706	NM_000900.1
203143_s_at	KIAA0040 gene product	Hs.158282	T79953
203892_at	WAP four-disulfide core domain 2	Hs.2719	NM_006103.1
203917_at	coxsackie virus and adenovirus receptor	Hs.79187	NM_001338.1
204942_s_at	aldehyde dehydrogenase 3 family, member B2	Hs.87539	NM_000695.2
205381_at	37 kDa leucine-rich repeat (LRR) protein	Hs.155545	NM_005824.1
205590_at	RAS guanyl releasing protein 1 (calcium and DAG-regulated)	Hs.182591	NM_005739.2
208798_x_at	golgin-67	Hs.182982	AF204231.1
209189_at	v-fos FBJ murine osteosarcoma viral oncogene homolog	Hs.25647	BC004490.1
212708_at	Homo sapiens mRNA; cDNA DKFZp586B1922 (from clone DKFZp586B1922)	Hs.184779	AV721987
212927_at	KIAA0594 protein	Hs.103283	AB011166.1
213089_at	ESTs, Highly similar to T17212 hypothetical protein DKFZp434P211.1 [H.sapiens]	Hs.352339	AU158490
213605_s_at	Homo sapiens mRNA; cDNA DKFZp564F112 (from clone DKFZp564F112)	Hs.166361	AL049987.1
214020_x_at	integrin, beta 5	Hs.149846	AI335208

214053_at	Homo sapiens clone 23736 mRNA sequence	Hs.7888	AW772192
214218_s_at	Homo sapiens cDNA FLJ30298 fis, clone BRACE2003172	Hs.351546	AV699347
214657_s_at	multiple endocrine neoplasia I	Hs.240443	AU134977
214705_at	PDZ domain protein (Drosophila inaD-like)	Hs.321197	AJ001306.1
215071_s_at	H2A histone family, member L	Hs.28777	AL353759
215470_at	Human chromosome 5q13.1 clone 5G8 mRNA	Hs.14658	U21915.1
217838_s_at	RNB6	Hs.241471	NM_016337.1
218312_s_at	hypothetical protein FLJ12895	Hs.235390	NM_023926.1
218330_s_at	retinoic acid inducible in neuroblastoma	Hs.23467	NM_018162.1
218344_s_at	hypothetical protein FLJ10876	Hs.94042	NM_018254.1
218398_at	mitochondrial ribosomal protein S30	Hs.28555	NM_016640.1

Claims

1. A method of creating an expression profile characteristic of a breast tumour cell, said method comprising the steps of

(a) isolating expression products from said breast tumour cell and a normal breast cell;

(b) contacting said expression products for both the tumour and normal breast cell with a plurality of binding members capable of specifically binding to expression products of one or more of the genes selected from Table 2; so as to create an expression profile of those genes for both the tumour cell and the normal cell;

(c) comparing the expression profile of the tumour cell and the normal cell; and

(d) determining an expression profile characteristic of a breast tumour cell.

2. A method of creating an expression profile characteristic of a breast tumour cell, said method comprising the steps of

(a) isolating expression products from a breast tumour cell, contacting said expression products with a plurality of binding members capable of specifically and independently binding to expression products of a plurality of genes selected from Table 2; so as to create a first expression profile of a tumour cell;

(b) isolating expression products from a normal breast cell; contacting said expression products with the plurality of binding members as used in step (a), so as to create a comparable second expression profile of a normal breast cell; and

(c) comparing the first and second expression profiles to determine an expression profile characteristic of a breast tumour cell.

3. A method of creating a nucleic acid expression profile characteristic of a breast tumour cell, said method comprising the steps of

(a) isolating expression products from a first breast tumour cell, contacting said expression products with a plurality of binding members capable of specifically and independently binding to expression products of a plurality of genes selected from Table 2, so as to create a first expression profile;

(b) repeating step (a) with expression products from at least a second breast tumour cell so as to create at least a second expression profile;

(c) comparing the at least first and second expression profiles to create a standard nucleic acid expression profile characteristic of a breast tumour cell.

4. A method according to any one of the preceding claims wherein the binding members are capable of specifically and independently binding to five or more genes selected from Table 2.

5. A method according to any one of the preceding claims wherein the binding members are capable of specifically and independently binding to each of the genes provided in Table 2.

6. A method according to any one of the preceding claims wherein the expression product is mRNA or cDNA.

7. A method according to any one of the preceding claims wherein the binding members are nucleic acid probes.

8. A method according to any one of claims 1 to 5 wherein the expression product is a polypeptide.

9. A method according to claim 8 wherein the binding members are antibody binding domains.

10. A method according to any one of the preceding claims wherein the binding members are labelled.

11. A method according to any one of claims 1 to 9 wherein the expression products are labelled.

12. A method for determining the presence or risk of breast cancer in an individual, said method comprising

(a) obtaining expression products from a breast tissue cell obtained from an individual suspected of having or at risk from having breast cancer;

(b) contacting said expression products with binding members capable of specifically and independently binding to expression products corresponding to a plurality of the genes identified in Table 2; and

(c) determining the presence or risk of breast cancer in said individual based on the binding of the expression products from said breast tissue cell to one or more of the binding members.

13. A method according to claim 12 wherein the binding members are capable of binding to expression products corresponding to at least five of the genes identified in Table 2.

14. A method according to claim 12 or claim 13 wherein the binding members are capable of binding to expression products corresponding to each of the genes identified in Table 2.

15. A method according to any one of claims 12 to 14 wherein the determination of the presence or risk of breast cancer in said individual is carried out by comparing the binding of the expression products from the breast tissue cell under test with an expression profile characteristic of breast tumour cell.

16. A method according to claim 15 wherein said expression profile characteristic of a breast tumour cell is created by a method according to any one of claims 1 to 11.

17. A method according to any one of claims 12 to 16 wherein the individual is of Asian descent.

18. A method of creating a nucleic acid expression profile characteristic of a breast tumour cell, said method comprising the steps of

(a) isolating expression products from said breast tumour cell and a normal breast cell;

(b) contacting said expression products for both the tumour and normal breast cell with a plurality of binding members capable of specifically binding to expression products of a plurality of genes selected from Table 4a; so as to create an expression profile of those genes for both the tumour cell and the normal cell;

(c) comparing the expression profile of the tumour cell and the normal cell; and

(d) determining a nucleic acid expression profile characteristic of breast tumour cell.

19. A method of creating a nucleic acid expression profile characteristic of a breast tumour cell, said method comprising the steps of

(a) isolating expression products from a breast tumour cell; contacting said expression products with a plurality of binding members capable of specifically and independently binding to expression products of a plurality of genes selected from Table 4a; so as to create a first expression profile of a tumour cell;

(b) isolating expression products from a normal breast cell; contacting said expression products with the plurality of binding members as used in step (a); so as to create a comparable second expression profile of a normal breast cell;

(c) comparing the first and second expression profiles to determine an expression profile characteristic of a breast tumour cell.

20. A method according to claim 18 or claim 19 wherein the said plurality of genes are selected from Table 4b.

21. A method according to claim 19 wherein at least five genes are selected from Table 4a.

22. A method according to claim 19 wherein at least twenty genes are selected from Table 4a.

23. A method according to claim 19 wherein the plurality of genes comprise at least those provided in Table 4b.

24. A method according to any one of claims 18 to 23 wherein the expression product is mRNA or cDNA.

25. A method according to any one of claims 18 to 23 wherein the binding members are nucleic acid probes.

26. A method according to any one of claims 18 to 23 wherein the expression product is a polypeptide.

27. A method according to claim 26 wherein the binding members are antibody binding domains.

28. A method according to any one of claims 18 to 27 wherein the binding members are labelled.

29. A method according to any one of claims 18 to 27 wherein the expression products are labelled.

30. A method for determining the presence or risk of breast cancer in an individual, said method comprising

(a) obtaining expression products from a breast tissue cell obtained from an individual suspected of having or at risk from having breast cancer;

(b) contacting said expression products with binding members capable of binding to expression products corresponding to a plurality of genes identified in Table 4a; and

(c) determining the presence or risk of breast cancer in said individual based on the binding of the expression products from said breast tissue cell to one or more of the binding members.

31. A method according to claim 30 wherein at least five genes are selected from Table 4a.

32. A method according to claim 30 wherein at least twenty genes are selected from Table 4a.

33. A method according to claim 23 wherein the plurality of genes are at least those identified in Table 4b.

34. A method according to any one of claims 30 to 33 or claim 24 wherein the determination of the presence or risk of breast cancer in said individual is carried out by comparing the binding of the expression products from the breast tissue cell under test with an expression profile characteristic of breast tumour cell.

35. A method according to claim 34 wherein said expression profile characteristic of a breast tumour cell is created by a method according to any one of claims 18 to 29.

36. A method according to any one of claims 30 to 35 wherein the determination of the presence or risk of

breast cancer is computed using an algorithm which distinguishes a tumour cell from normal cell by their respective expression profiles.

37. A method of obtaining a plurality of gene expression profiles in order to determine a standard expression profile characteristic of presence and/or type of breast cancer, said method comprising

a) obtaining cells from a plurality of breast tumour sample;

b) disrupting said cells to expose gene expression products;

c) contacting said gene expression products with a plurality of binding members specific for expression products of one or more genes selected from Table 2; and

d) determining a gene expression profile characteristic of the presence and/or type of breast cancer based on the binding of said expression products to said binding members for each of said plurality of breast tumour samples.

38. A method of obtaining a plurality of gene expression profiles in order to determine a standard expression profile characteristic of presence and/or type of breast cancer, said method comprising

a) obtaining cells from a plurality of breast tumour sample;

b) disrupting said cells to expose gene expression products;

c) contacting said gene expression products with a plurality of binding members specific for expression products of one or more genes selected from Table 4a; and

d) determining a gene expression profile characteristic of the presence and/or type of breast cancer based on the binding of said expression products to said binding members for each of said plurality of breast tumour samples.

39. A method of obtaining a plurality of gene expression profiles in order to determine a standard expression profile characteristic of presence and/or type of breast cancer, said method comprising

a) obtaining cells from a plurality of breast tumour sample;

b) disrupting said cells to expose gene expression products;

c) contacting said gene expression products with a plurality of binding members specific for expression products of one or more genes selected from Table 4b; and

d) determining a gene expression profile characteristic of the presence and/or type of breast cancer based on the binding of said expression products to said binding members for each of said plurality of breast tumour samples.

40. A method of obtaining a plurality of gene expression profiles in order to determine a standard expression profile characteristic of presence and/or type of breast cancer, said method comprising

a) obtaining cells from a plurality of breast tumour sample;

b) disrupting said cells to expose gene expression products;

c) contacting said gene expression products with a plurality of binding members specific for expression products of one or more genes selected from Table 5; and

d) determining a gene expression profile characteristic of the presence and/or type of breast cancer based on the binding of said expression products to said binding members for each of said plurality of breast tumour samples.

41. A method of obtaining a plurality of gene expression profiles in order to determine a standard expression profile characteristic of presence and/or type of breast cancer, said method comprising

a) obtaining cells from a plurality of breast tumour sample;

b) disrupting said cells to expose gene expression products;

c) contacting said gene expression products with a plurality of binding members specific for expression products of one or more genes selected from Table 6a; and

d) determining a gene expression profile characteristic of the presence and/or type of breast cancer based on the binding of said expression products to said binding members for each of said plurality of breast tumour samples.

42. A method of obtaining a plurality of gene expression profiles in order to determine a standard expression profile characteristic of presence and/or type of breast cancer, said method comprising

a) obtaining cells from a plurality of breast tumour sample;

b) disrupting said cells to expose gene expression products;

c) contacting said gene expression products with a plurality of binding members specific for expression products of one or more genes selected from Table 7; and

d) determining a gene expression profile characteristic of the presence and/or type of breast cancer based on the binding of said expression products to said binding members for each of said plurality of breast tumour samples.

43. A method of obtaining a plurality of gene expression profiles in order to determine a standard expression profile characteristic of presence and/or type of breast cancer, said method comprising

a) obtaining cells from a plurality of breast tumour sample;

b) disrupting said cells to expose gene expression products;

c) contacting said gene expression products with a plurality of binding members capable of specifically and independently binding to expression products of the genes identified in Table 6b;

d) determining a gene expression profile characteristic of the presence and/or type of breast cancer based on the binding of said expression products to said binding members for each of said plurality of breast tumour samples.

44. A method according to any one of claims 37 to 43 further comprising the step of producing a database

containing a plurality of expression profiles obtained from said plurality of breast tumour samples.

45. A method according to any one of claims 37 to 43 further comprising the step of determining the statistical variation between the plurality of expression profiles.

46. A database comprising expression profiles characteristic of breast cancer or type of breast cancer produced by a method according to claim 37 or claim 45.

47. A database according to claim 46 wherein the expression profiles are nucleic acid expression profiles.

48. A database according to claim 46 wherein the expression profiles are protein expression profiles.

49. A method for classifying a breast tumour cell on the basis of Estrogen receptor (ER) status, said method comprising

(a) obtaining expression products from a breast tumour cell;

(b) contacting said expression products with binding members capable of binding to expression products corresponding to the genes identified in Table 5a; and

(c) classifying the breast tumour on the basis of ER status based on the binding of the expression products from said breast tumour cell to one or more of the binding members.

50. A method for classifying a breast tumour cell on the basis of ERBB2 status, said method comprising

(a) obtaining expression products from a breast tumour cell;

(b) contacting said expression products with binding members capable of binding to expression products corresponding to the genes identified in Table 5b; and

(c) classifying the breast tumour on the basis of ERBB2 status based on the binding of the expression products from said breast tumour cell to one or more of the binding members.

51. A method for classifying a breast tumour cell on the basis of its molecular subtype, said method comprising

(a) obtaining expression products from a breast tumour cell;

(b) contacting said expression products with binding members capable of binding to expression products corresponding to a plurality of genes identified in Table 6a; and

(c) classifying the tumour cell with regard to its molecular subtype based on the binding profile of the expression products from the tumour cell and the binding members.

52. A method according to claim 51 wherein the binding members are capable of specifically and independently binding to at least 5 genes identified in Table 6a.

53. A method according to claim 51 wherein the binding members are capable of specifically and independently binding to at least twenty genes identified in Table 6a.

54. A method according to claim 51 wherein the binding members are capable of specifically and independently binding to at least the genes identified in Table 6b.

55. A method according to any one of claims 51 to 54 wherein the molecular subtypes are selected from Luminal, ERBB2, Basal, ER-type II and normal/normal-like.

56. A method for classifying a breast tumour cell on the basis of its Luminal sub-class, said method comprising

(a) obtaining expression products from a breast tumour cell;

(b) contacting said expression products with binding members capable of binding to expression products corresponding to a plurality of genes identified in Table 7; and

(c) classifying the tumour cell with regard to its Luminal sub-class based on the binding profile of the expression products from the tumour cell and the binding members.

57. A method according to claim 56 wherein said tumour cell has been previously classified as a Luminal molecular subtype by a method according to any one of claims 51 to 55.

58. A method according to claim 56 or claims 57 wherein the Luminal sub-class is Luminal D or Luminal A.

59. A diagnostic tool comprising a plurality of binding members capable of specifically and independently binding

to expression products of a plurality of genes selected from Table 4a, said plurality of binding members being fixed to a solid support.

60. A diagnostic tool comprising a plurality of binding members capable of specifically and independently binding to expression products of a plurality of genes selected from Table 4b, said plurality of binding members being fixed to a solid support.

61. A diagnostic tool comprising a plurality of binding members capable of specifically and independently binding to expression products of a plurality of genes selected from Table 5a, said plurality of binding members being fixed to a solid support.

62. A diagnostic tool comprising a plurality of binding members capable of specifically and independently binding to expression products of a plurality of genes selected from Table 5b, said plurality of binding members being fixed to a solid support.

63. A diagnostic tool comprising a plurality of binding members capable of specifically and independently binding to expression products of a plurality of genes selected from Table 6a, said plurality of binding members being fixed to a solid support.

64. A diagnostic tool comprising a plurality of binding members capable of specifically and independently binding to expression products of a plurality of genes selected

from Table 7, said plurality of binding members being fixed to a solid support.

65. A diagnostic tool comprising a plurality of binding members capable of specifically and independently binding to expression products of the genes identified in Table 6b, said plurality of binding members being fixed to a solid support.

66. A diagnostic tool according to any one of claims 59 to 65 wherein said binding members are cDNA or oligonucleotides.

Fig.1.

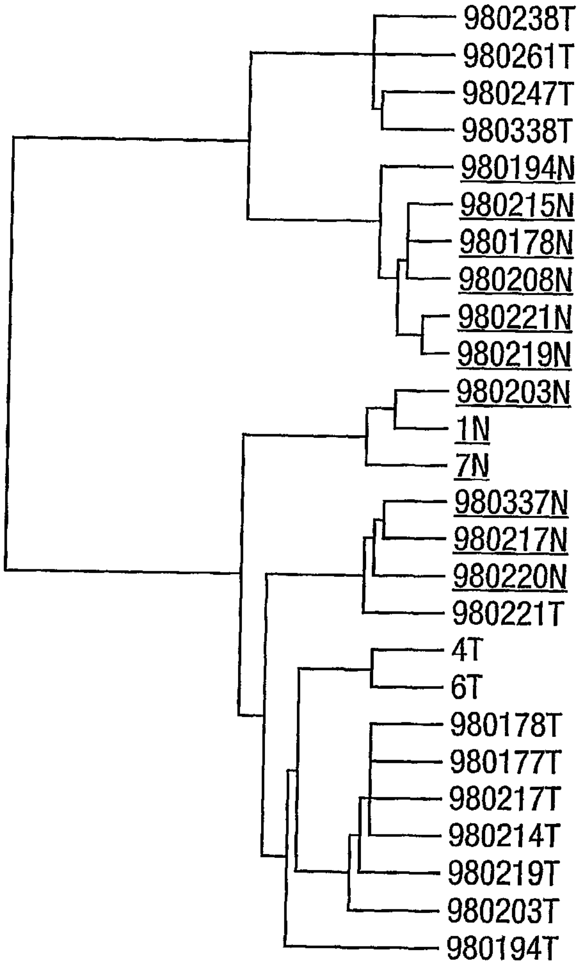

980238T
980261T
980247T
980338T
980194N
980215N
980178N
980208N
980221N
980219N
980203N
1N
7N
980337N
980217N
980220N
980221T
4T
6T
980178T
980177T
980217T
980214T
980219T
980203T
980194T

Fig.2(A).

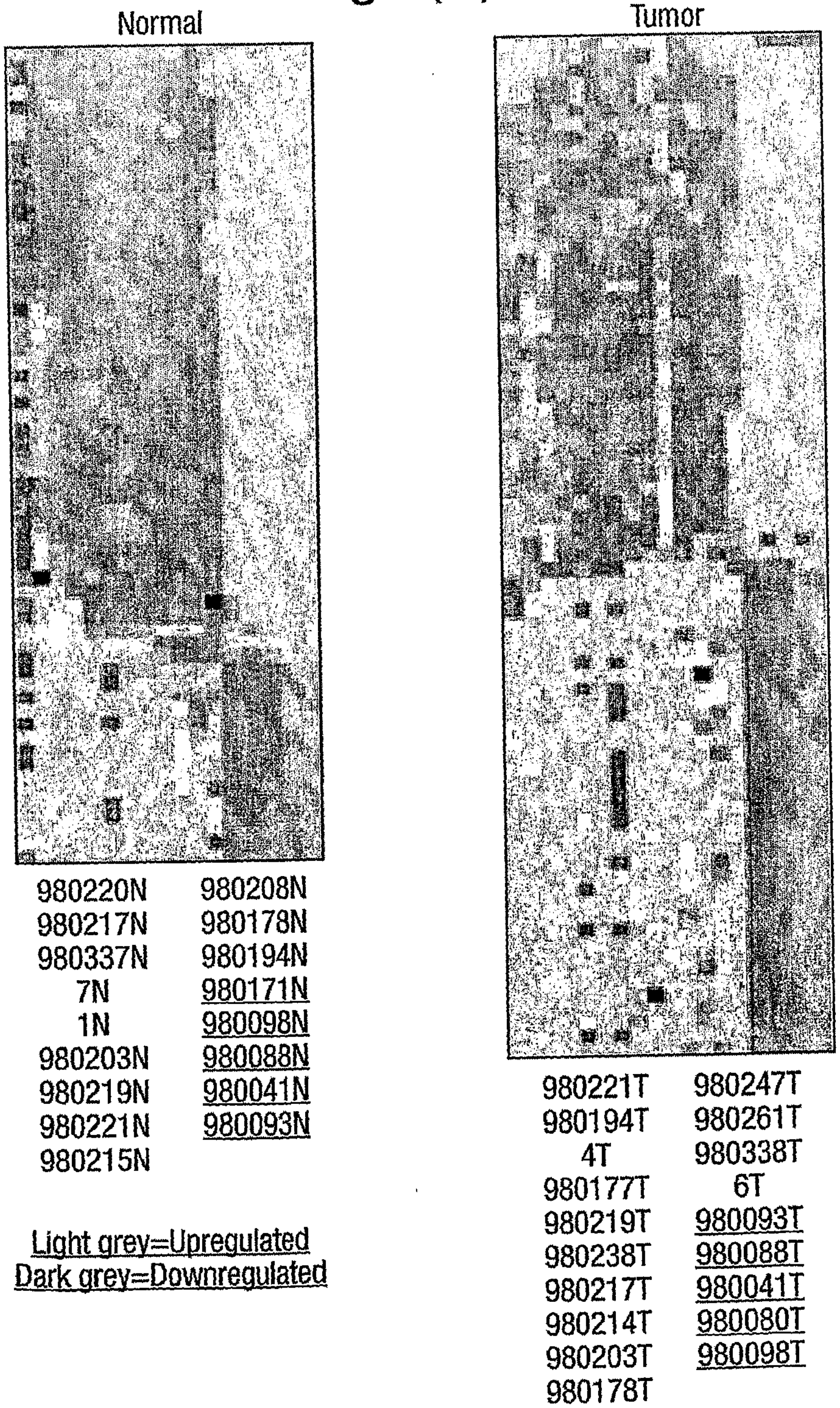
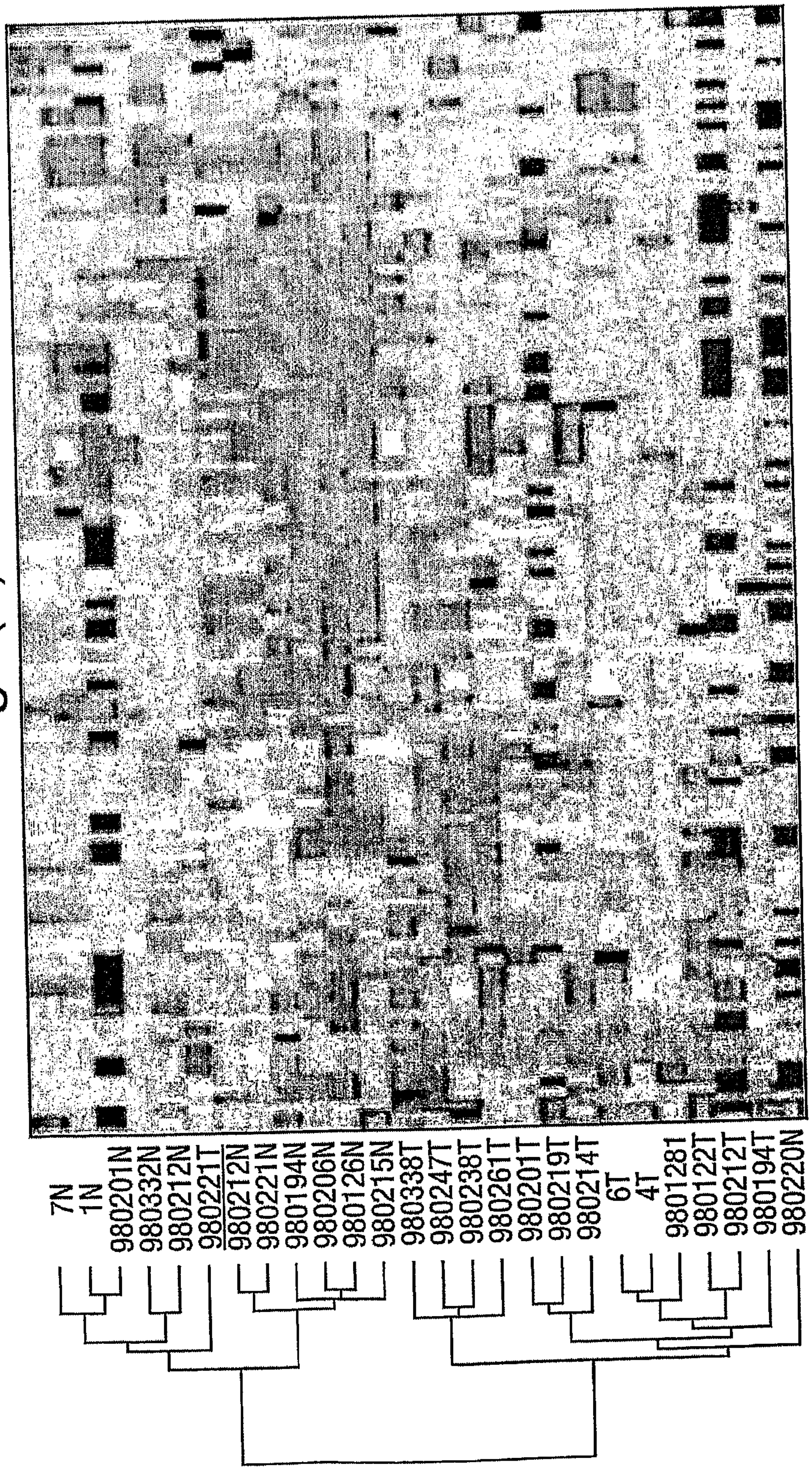

Normal
Tumor
980220N
980217N
980337N
7N
1N
980203N
980219N
980221N
980215N
980208N
980178N
980194N
980171N
980098N
980088N
980041N
980093N
980221T
980194T
4T
980177T
980219T
980238T
980217T
980214T
980203T
980178T
980247T
980261T
980338T
6T
980093T
980088T
980041T
980080T
980098T
Light grey=Upregulated
Dark grey=Downregulated

Fig.2(B).



Light grey=Upregulated
Dark grey=Downregulated

Fig.3(A).

Test Set (26 Samples)

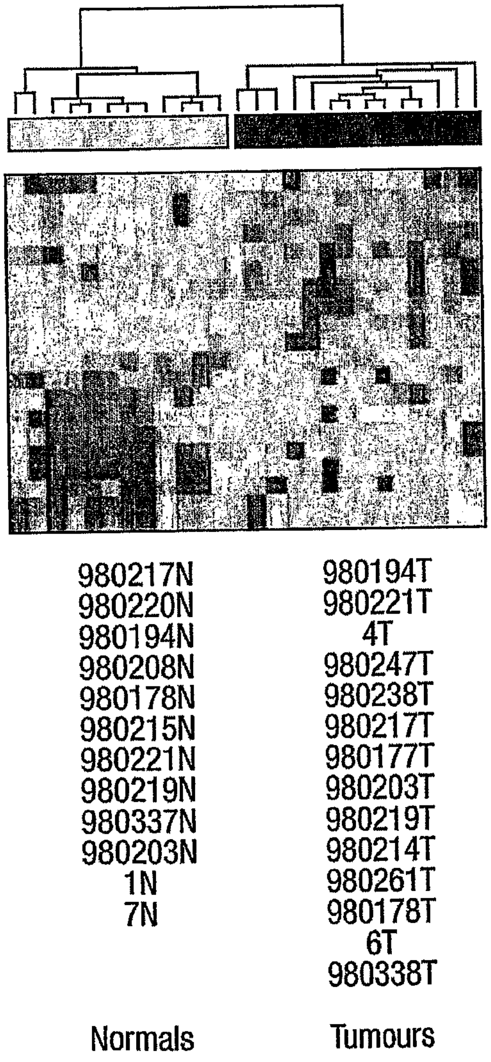


Fig.3(B).

Test Set (22 Samples)

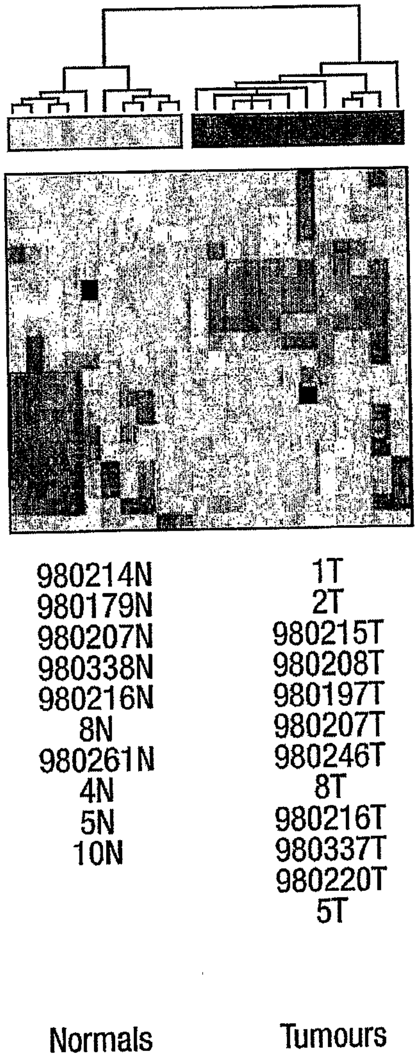
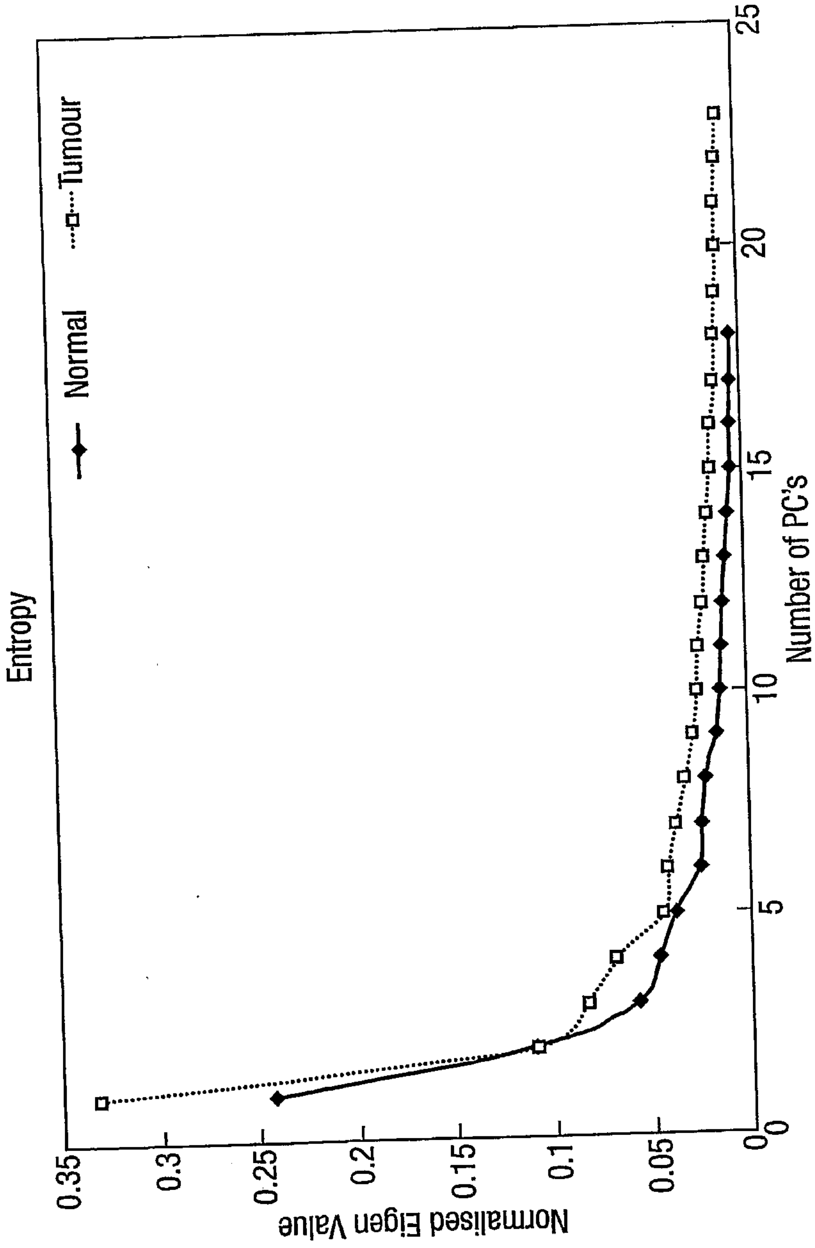


Fig.4.


Entropy
Normalised Eigen Value
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Number of PC's
0
5
10
15
20
25
Normal
Tumour

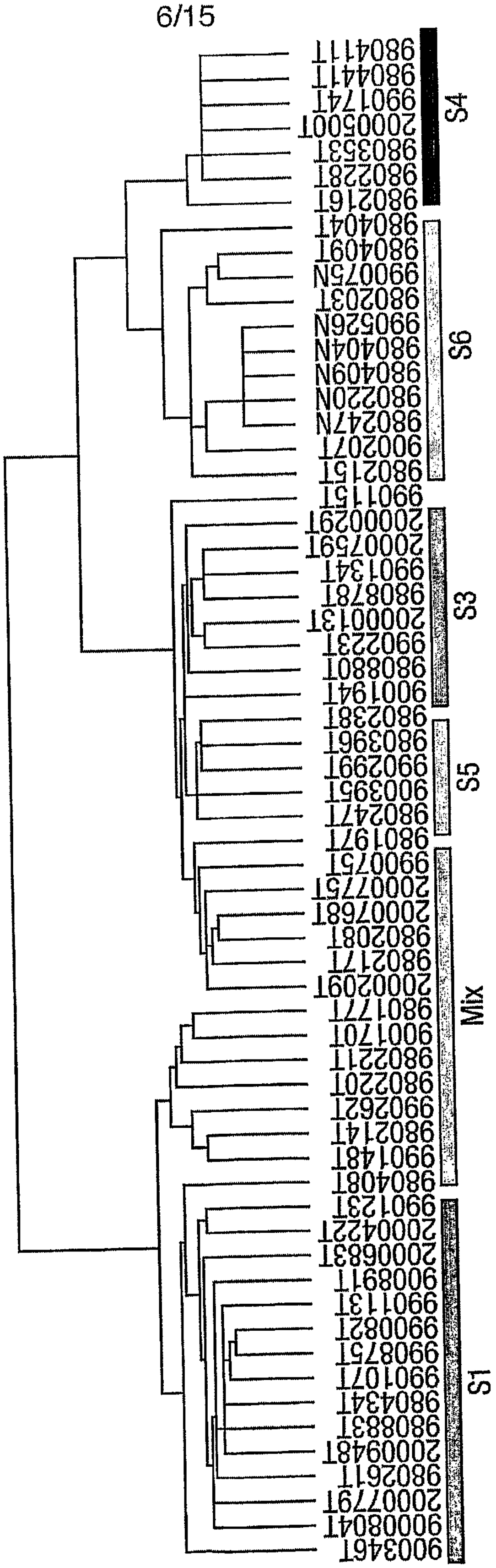

980411T
980441T
990174T
2000500T
980353T
980228T
980216T
980404T
980409T
990075N
980203T
990526N
980404N
980409N
980220N
980247N
900207T
980215T
990115T
2000029T
2000759T
990134T
980878T
2000013T
990223T
980880T
900194T
980238T
980396T
990299T
900395T
980247T
980197T
990075T
2000775T
2000768T
980208T
980217T
2000209T
980177T
900170T
980221T
980220T
990262T
980214T
990148T
980408T
990123T
2000422T
2000683T
900891T
990113T
990082T
990875T
990107T
980434T
980883T
2000948T
980261T
2000779T
900804T
900346T
S4
S6
S3
S5
Mix
S1

Fig.5(A).

Fig.5(B).

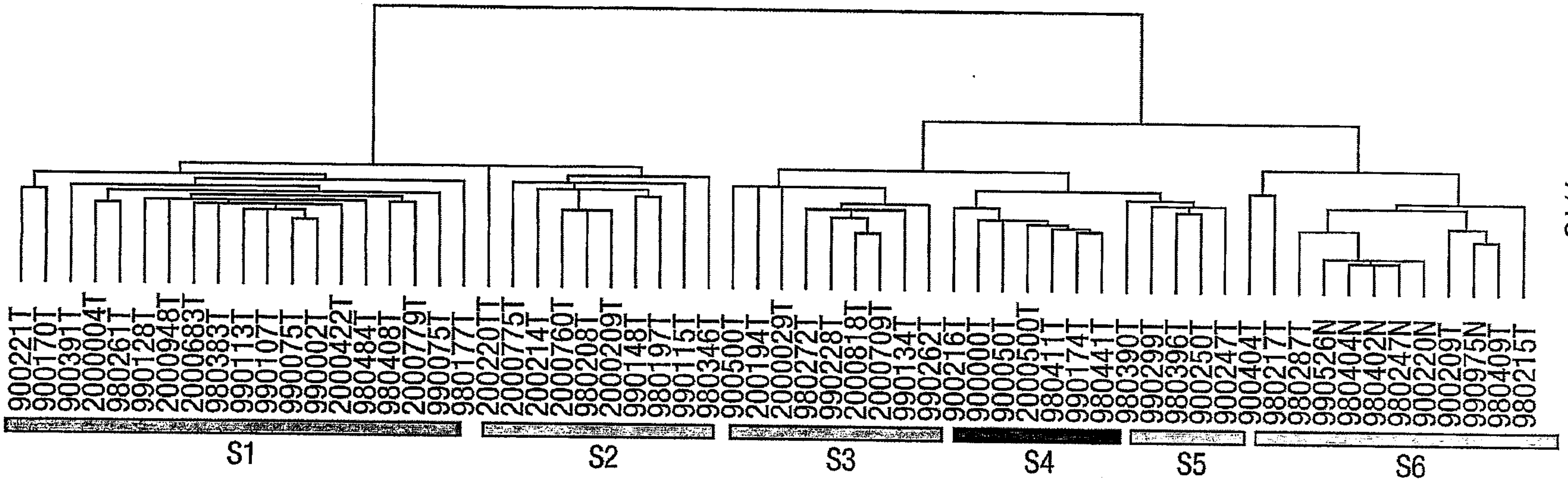

900221T
900170T
900391T
2000004T
980261T
990128T
2000948T
2000683T
980383T
990113T
990107T
990075T
990002T
2000422T
980484T
980408T
2000779T
990075T
980177T
200220TT
2000775T
200214T
2000760T
980208T
2000209T
990148T
980197T
990115T
980346T
900500T
200194T
2000029T
980272T
990228T
2000818T
2000709T
990134T
990262T
900216T
900000T
900050T
2000500T
980411T
990174T
980441T
980390T
990299T
980396T
900250T
900247T
900404T
980217T
980287T
990526N
980404N
980402N
980247N
900220N
900209T
990975N
980409T
980215T
S1
S2
S3
S4
S5
S6

Fig.5(C).

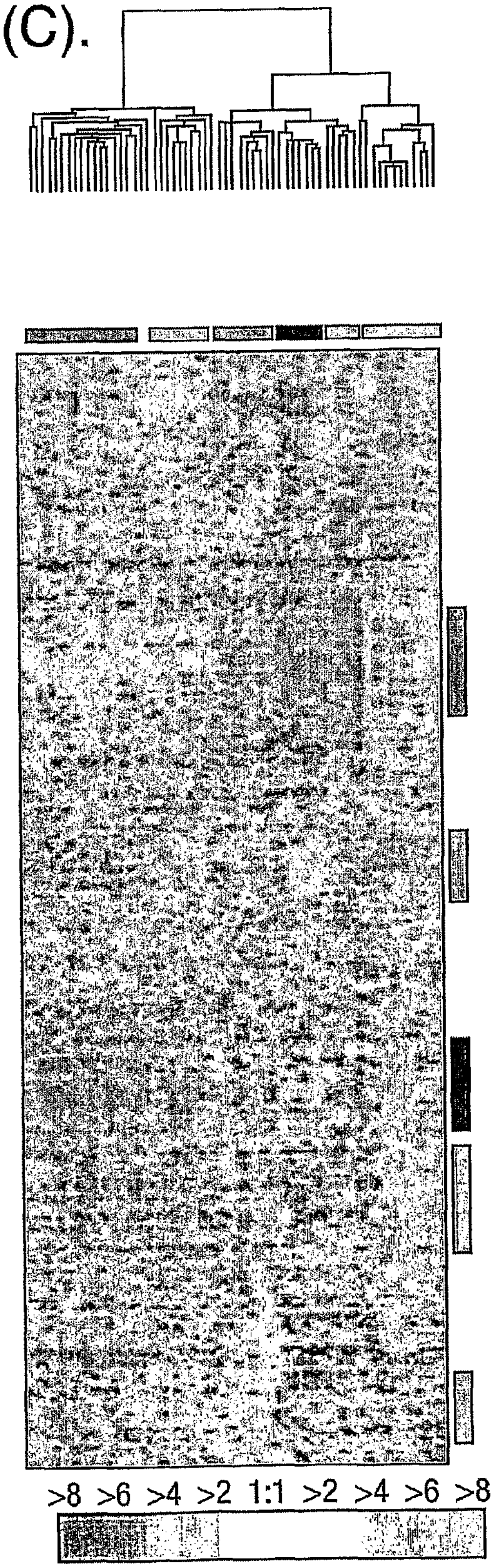
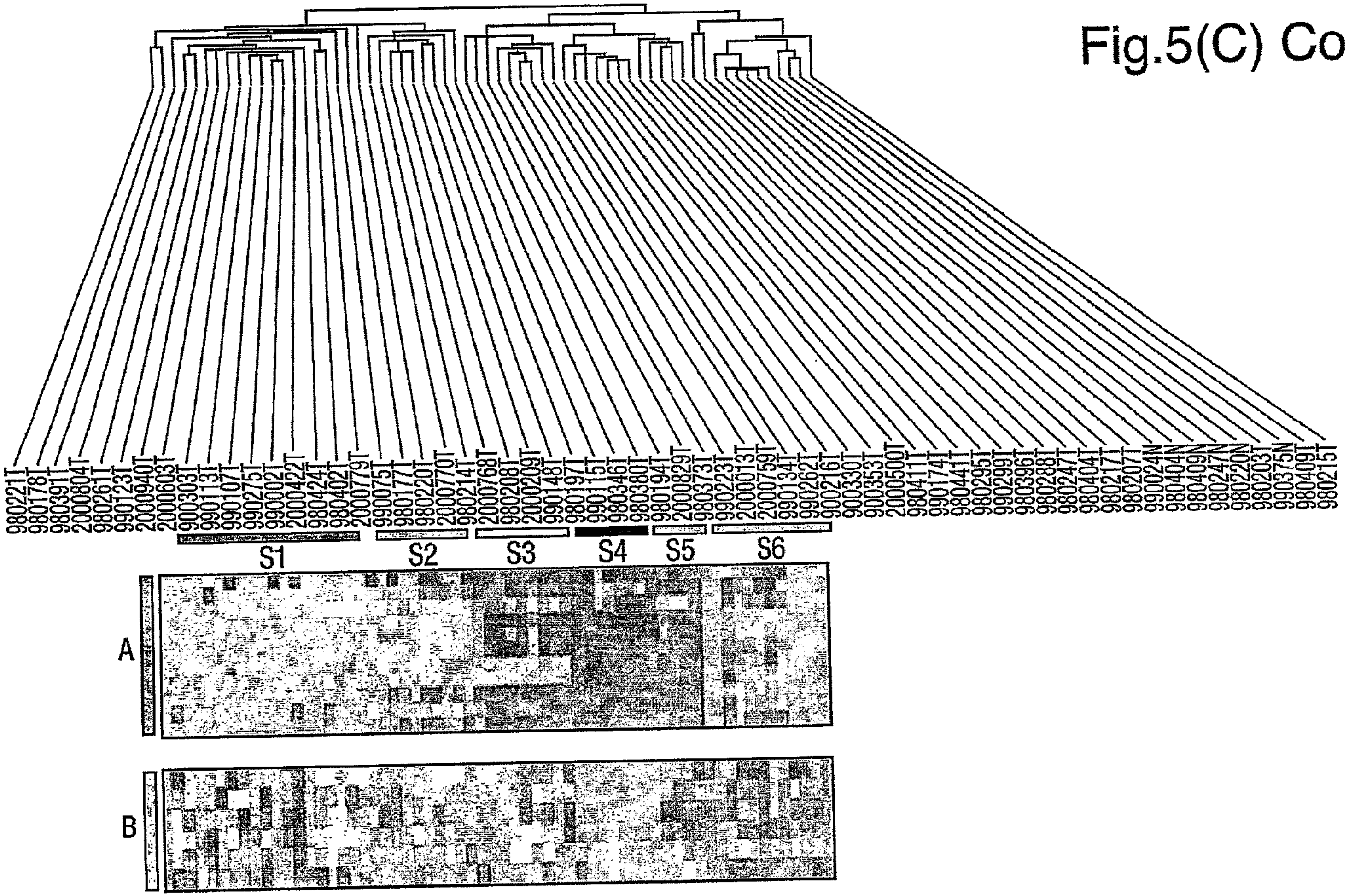


Fig.5(C) Cont I.


980221T
980178T
980391T
2000804T
980261T
990123T
2000940T
2000603T
900303T
990113T
990107T
990275T
990002T
2000422T
980424T
980402T
2000779T
990075T
980177T
980220T
2000770T
980214T
2000768T
980208T
2000209T
990148T
980197T
990115T
980346T
980380T
980194T
2000829T
980373T
990223T
2000013T
2000759T
990134T
990262T
900216T
900330T
900353T
2000500T
980411T
990174T
980441T
980295T
990299T
980396T
980288T
980247T
980404T
980217T
980207T
990024N
980404N
980409N
980247N
980220N
980203T
990375N
980409T
980215T
S1
S2
S3
S4
S5
S6
A
B

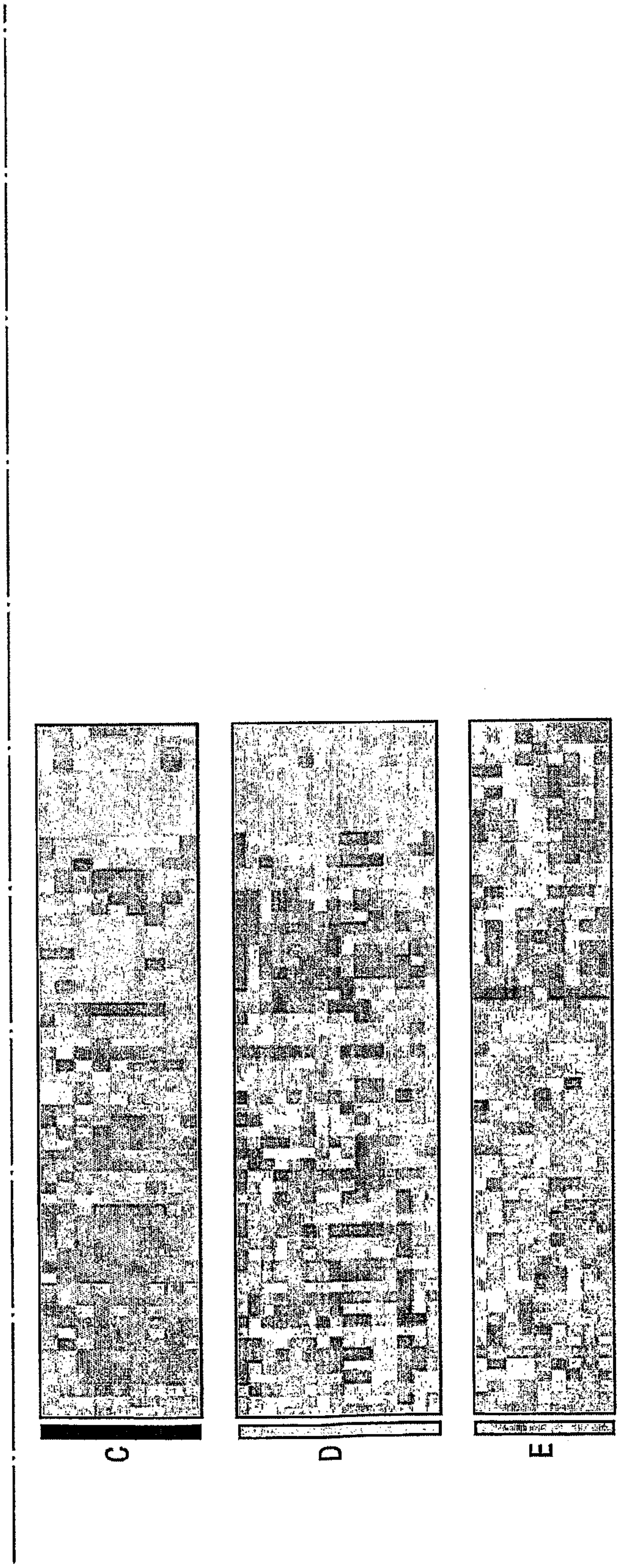


Fig.5(C) Cont II.

Fig.6(A).

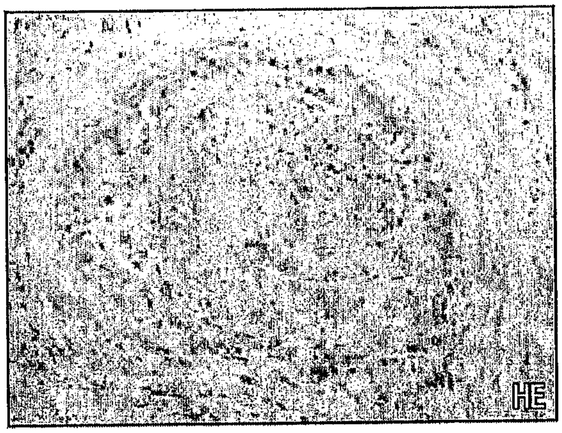


Fig.6(B).

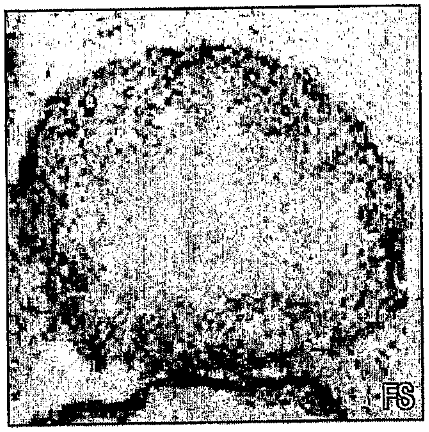


Fig.6(C).

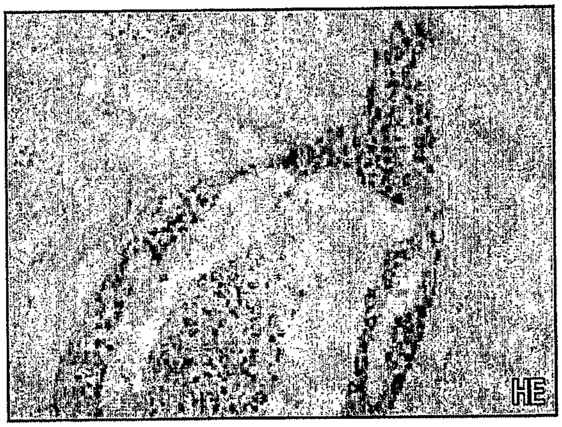


Fig.6(D).

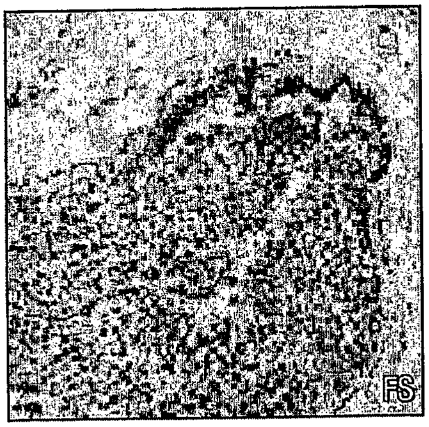
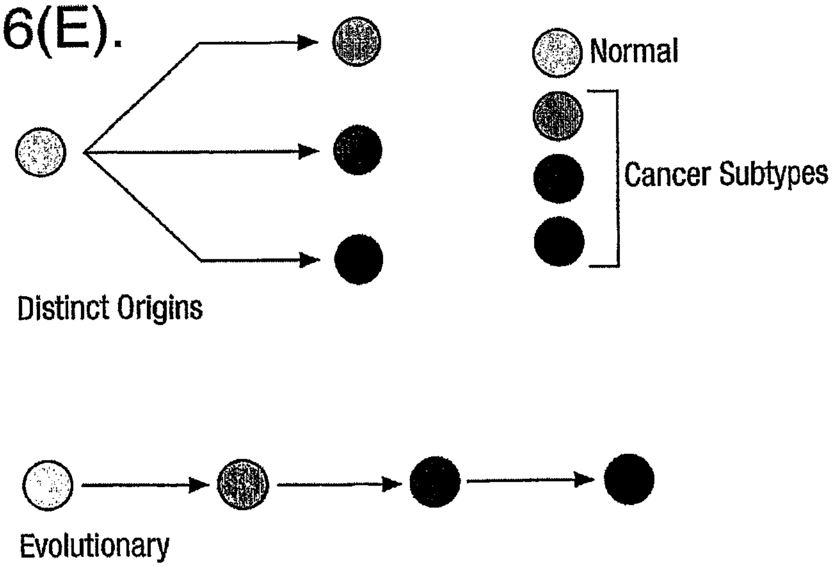


Fig.6(E).



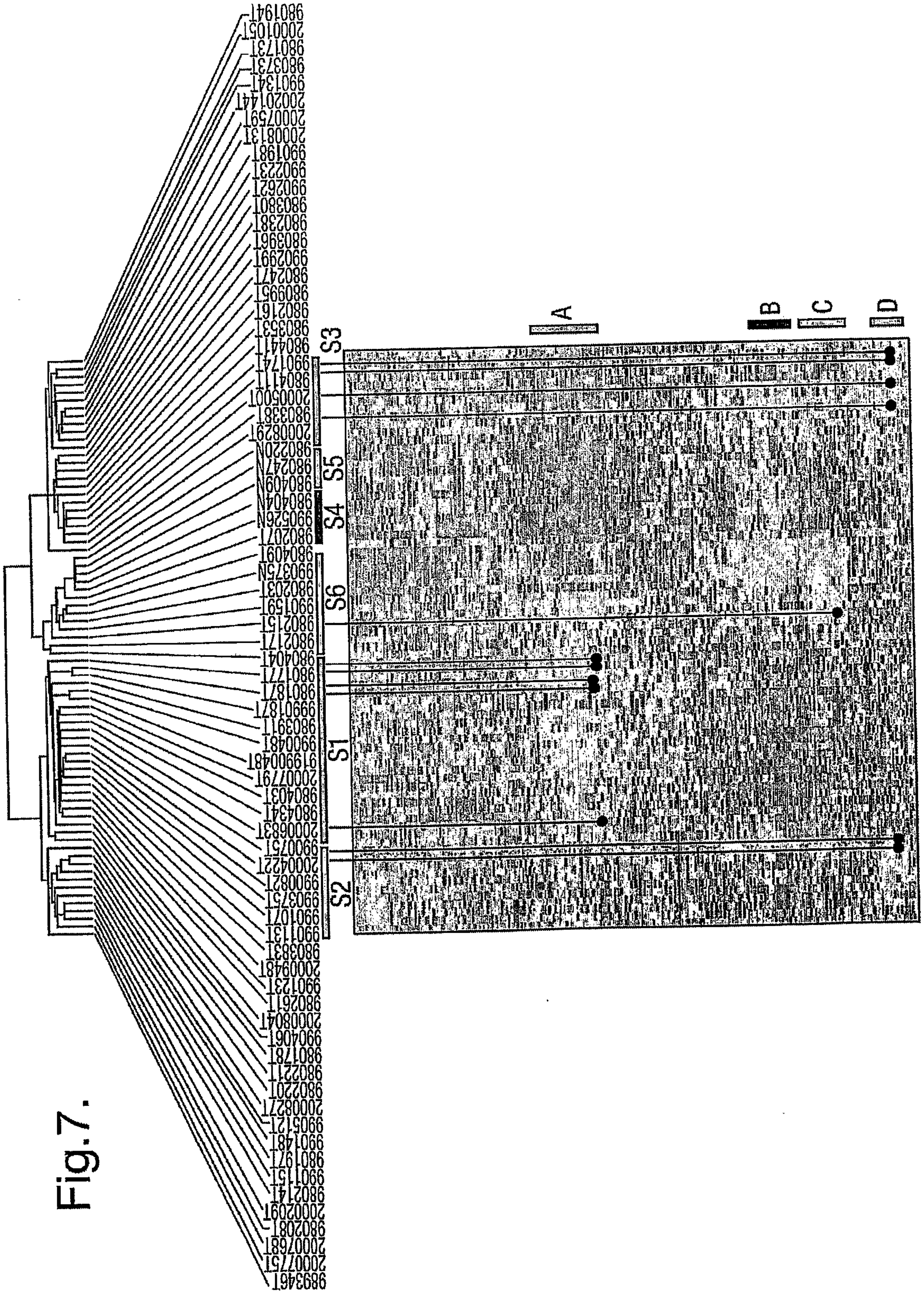

9801941T
20001051T
9801731T
9803731T
9901341T
20020144T
20007591T
20008131T
9901981T
9902231T
9902621T
9803801T
9802381T
9803961T
9902991T
9802471T
9809951T
9802161T
9803531T
9804411T
9901741T
9804111T
20005001T
9803381T
20006291T
980220N
980247N
980409N
980404N
990526N
9802071T
9804091T
990375N
9802031T
9901591T
9802151T
9802171T
9804041T
9801771T
9801871T
99901871T
9803911T
9900481T
919900481T
20007791T
9804031T
9804341T
20006831T
9900751T
20004221T
9900821T
9903751T
9901071T
9901131T
9803831T
20009481T
9901231T
9802611T
20008041T
9904061T
9801781T
9802211T
9802201T
20008271T
9905121T
9901481T
9801971T
9901151T
9802141T
20002091T
9802081T
20007681T
20007751T
9893461T
S3
S5
S4
S6
S1
S2
A
B
C
D

Fig.7.

Fig.8(A).

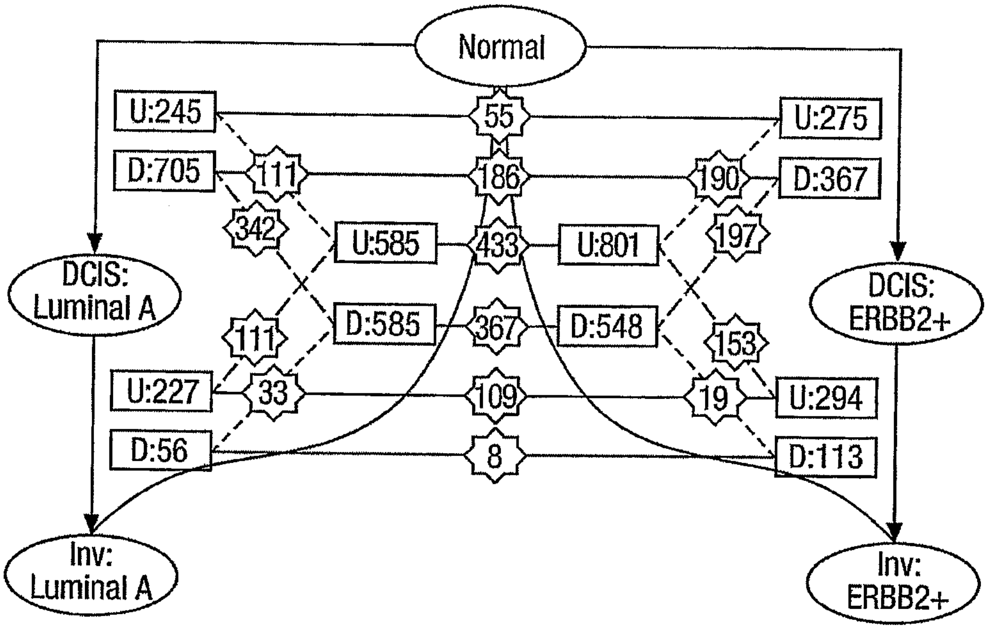

Normal
U:245
55
U:275
D:705
111
186
190
D:367
342
U:585
433
U:801
197
DCIS: Luminal A
DCIS: ERBB2+
111
D:585
367
D:548
153
U:227
33
109
19
U:294
D:56
8
D:113
Inv: Luminal A
Inv: ERBB2+

Fig.8(B).

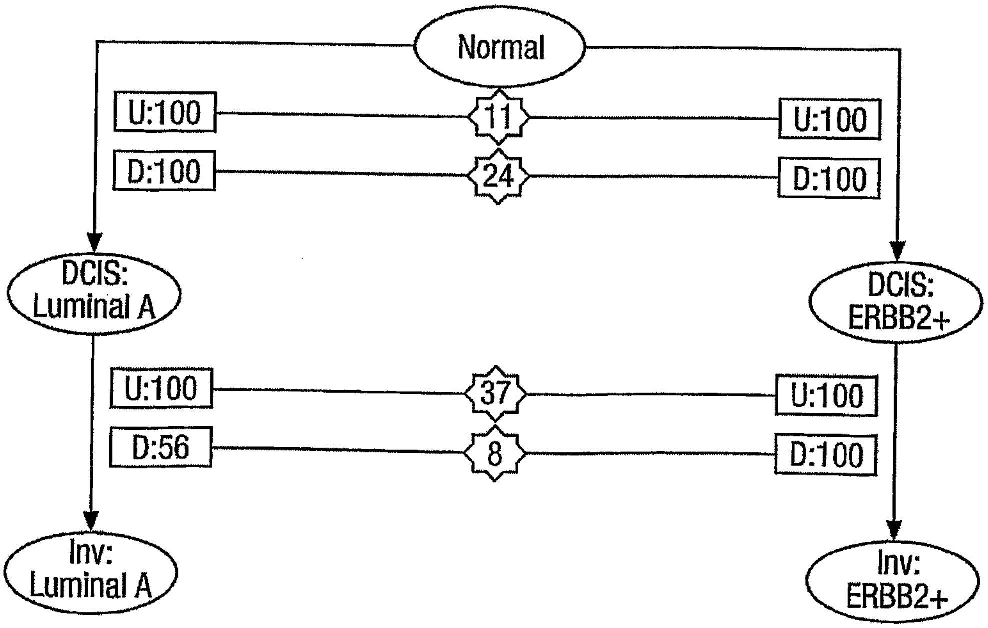

Normal
U:100
11
U:100
D:100
24
D:100
DCIS: Luminal A
DCIS: ERBB2+
U:100
37
U:100
D:56
8
D:100
Inv: Luminal A
Inv: ERBB2+

Fig.9(A).

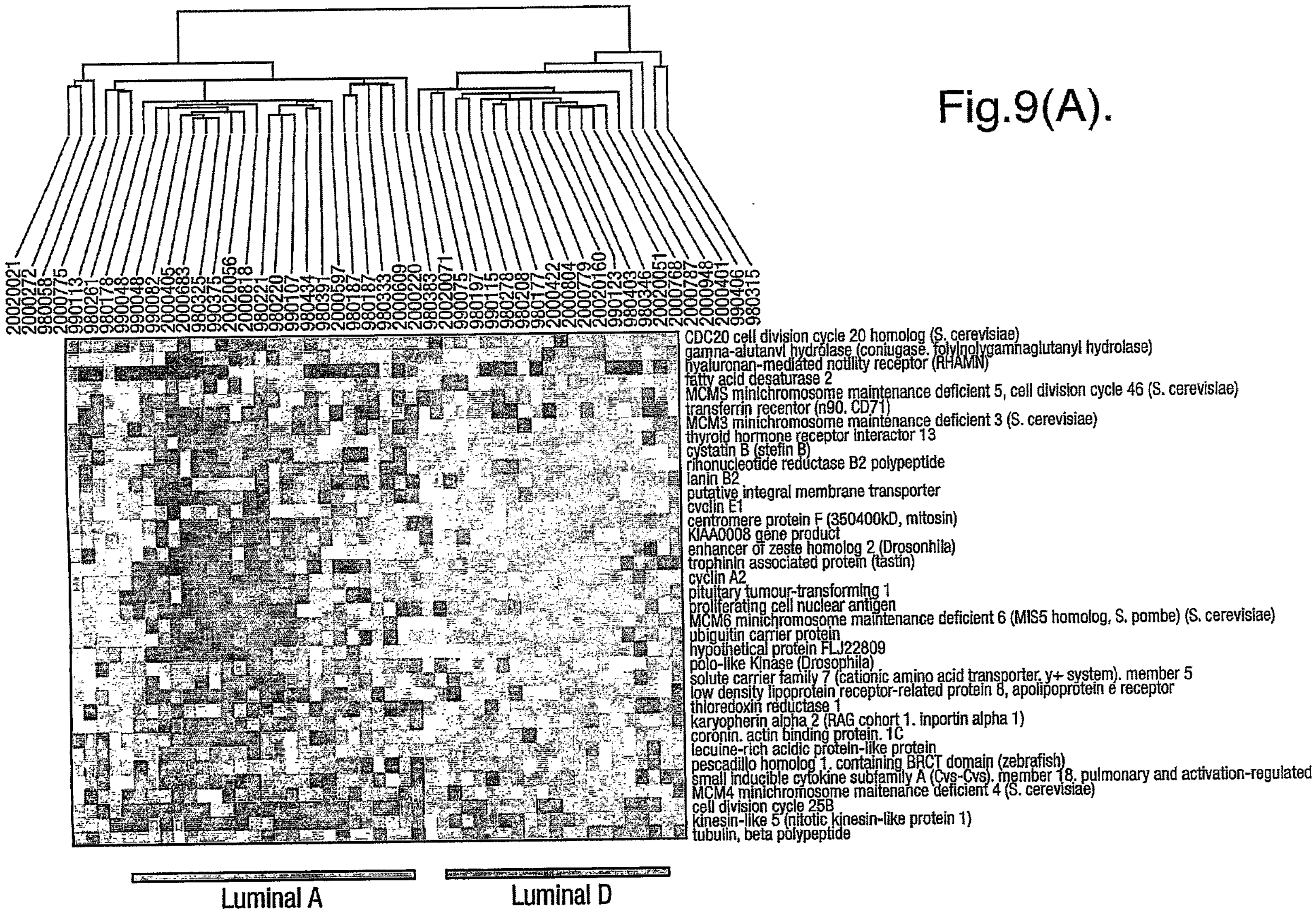
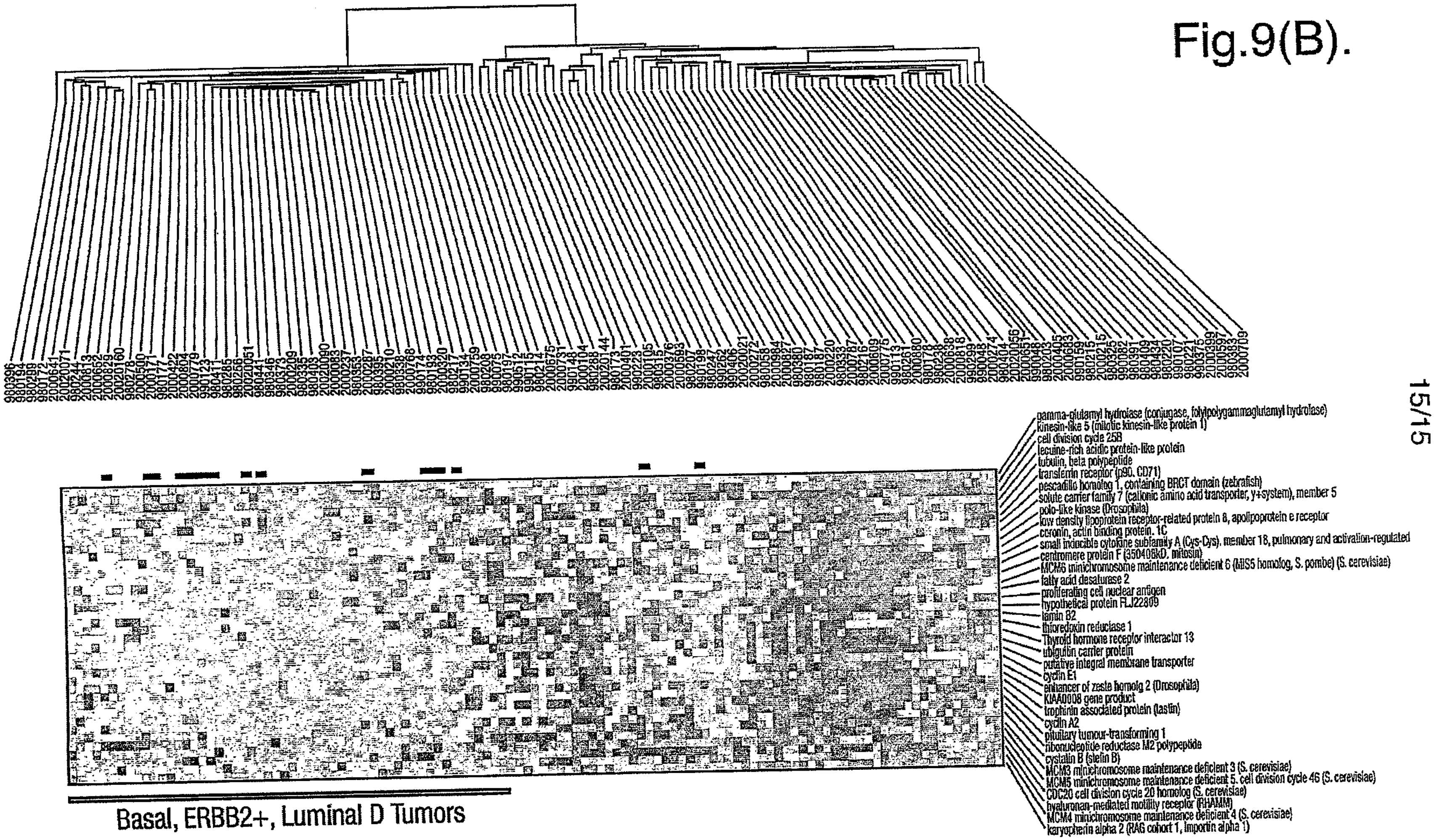

20020021
2000272
980058
2000775
990113
980261
980178
990048
990048
990082
2000405
2000683
980325
990375
20020056
2000818
980221
980220
990107
980434
980391
2000597
980187
980187
980333
2000609
2000220
980383
20020071
990075
980197
990115
980278
980208
980177
2000422
2000804
2000779
20020160
990123
980403
980346
20020051
2000768
2000787
2000948
2000401
990406
980315
CDC20 cell division cycle 20 homolog (S. cerevisiae)
gamna-alutanvl hydrolase (coniugase. folylnolygamnaglutanyl hydrolase)
hyaluronan-mediated notility receptor (RHAMN)
fatty acid desaturase 2
MCMS minichromosome maintenance deficient 5, cell division cycle 46 (S. cerevisiae)
transferrin recentor (n90. CD71)
MCM3 minichromosome maintenance deficient 3 (S. cerevisiae)
thyroid hormone receptor interactor 13
cystatin B (stefin B)
rihonucleotide reductase B2 polypeptide
lanin B2
putative integral membrane transporter
cvclin E1
centromere protein F (350400kD, mitosin)
KIAA0008 gene product
enhancer of zeste homolog 2 (Drosonhila)
trophinin associated protein (tastin)
cyclin A2
pituitary tumour-transforming 1
proliferating cell nuclear antigen
MCM6 minichromosome maintenance deficient 6 (MIS5 homolog, S. pombe) (S. cerevisiae)
ubiguitin carrier protein
hypothetical protein FLJ22809
polo-like Kinase (Drosophila)
solute carrier family 7 (cationic amino acid transporter. y+ system). member 5
low density lipoprotein receptor-related protein 8, apolipoprotein e receptor
thioredoxin reductase 1
karyopherin alpha 2 (RAG cohort 1. inportin alpha 1)
coronin. actin binding protein. 1C
lecuine-rich acidic protein-like protein
pescadillo homolog 1. containing BRCT domain (zebrafish)
small inducible cytokine subfamily A (Cvs-Cvs). member 18. pulmonary and activation-regulated
MCM4 minichromosome maitenance deficient 4 (S. cerevisiae)
cell division cycle 25B
kinesin-like 5 (nitotic kinesin-like protein 1)
tubulin, beta polypeptide
Luminal A
Luminal D

Fig.9(B).


980396
980194
980299
980172
2000641
20020071
980244
2000813
2000652
2000829
20020160
980278
2000500
2000171
980177
2000422
2000804
2000779
990123
980411
980285
980256
20020051
980441
980346
980373
2000209
980335
980403
20020090
2000083
2000237
980353
2000287
980395
2000210
980338
2000768
990174
980193
2000320
980217
990134
2000759
980208
990075
980197
990512
990115
980214
2000675
2000731
990148
2000104
980288
20020144
980173
2000401
990223
2000105
980315
2000376
2000593
980207
990198
980247
990262
990406
20020021
2000272
980058
2000984
2000827
980380
980187
980187
2000220
980333
2000787
980216
2000609
2000775
990113
980261
2000880
980178
980238
2000638
2000818
990299
990048
2000274
980404
20020056
2000651
990048
980203
2000405
2000683
990159
980215
2000215
980325
990082
980391
980409
980434
980220
990107
980221
990375
2000399
2000597
980383
2000709
gamma-glutamyl hydrolase (conjugase, folylpolygammaglutamyl hydrolase)
kinesin-like 5 (mitotic kinesin-like protein 1)
cell division cycle 25B
lecuine-rich acidic protein-like protein
tubulin, beta polypeptide
transferrin receptor (p90. CD71)
pescadillo homolog 1, containing BRCT domain (zebrafish)
solute carrier family 7 (cationic amino acid transporter, y+system), member 5
polo-like kinase (Drosophila)
low density lipoprotein receptor-related protein 8, apolipoprotein e receptor
coronin, actin binding protein. 1C
small inducible cytokine subfamily A (Cys-Cys). member 18, pulmonary and activation-regulated
centromere protein F (350408kD. mitosin)
MCM6 minichromosome maintenance deficient 6 (MIS5 homolog, S. pombe) (S. cerevisiae)
fatty acid desaturase 2
proliferating cell nuclear antigen
hypothetical protein FLJ22809
lamin B2
thioredoxin reductase 1
Thyroid hormone receptor interactor 13
ubiquitin carrier protein
putative integral membrane transporter
cyclin E1
enhancer of zeste homolg 2 (Drosophila)
KIAA0008 gene product
trophinin associated protein (tastin)
cyclin A2
pituitary tumour-transforming 1
ribonucleotide reductase M2 polypeptide
cystatin B (stefin B)
MCM3 minichromosome maintenance deficient 3 (S. cerevisiae)
MCM5 minichromosome maintenance deficient 5. cell division cycle 46 (S. cerevisiae)
CDC20 cell division cycle 20 homolog (S. cerevisiae)
hyaluronan-mediated motility receptor (RHAMM)
MCM4 minichromosome maintenance deficient 4 (S. cerevisiae)
karyopherin alpha 2 (RAG cohort 1, importin alpha 1)
Basal, ERBB2+, Luminal D Tumors

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
28 August 2003 (28.08.2003)

PCT

(10) International Publication Number
WO 2003/070979 A3

(51) International Patent Classification⁷: C12Q 1/68, G01N 33/574

(21) International Application Number: PCT/GB2003/000755

(22) International Filing Date: 20 February 2003 (20.02.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

0203998.0	20 February 2002 (20.02.2002)	GB
2002-130927	2 May 2002 (02.05.2002)	JP

(71) Applicant *(for all designated States except US)*: **NCC TECHNOLOGY VENTURES PTE LIMITED** [SG/SG]; 11 Hospital Drive, 169610 Singapore (SG).

(71) Applicant *(for MN only)*: **CRIPPS, Joanna, E.** [GB/GB]; Mewburn Ellis, 1 Redcliff Street, Bristol, Bristol BS1 6NP (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **TAN, Patrick** [SG/SG]; National Cancer Centre, 11 Hospital Drive, 169610 Singapore (SG). **KUN, Yu** [SG/SG]; National Cancer Centre, 11 Hospital Drive, 169610 Singapore (SG). **AGGARWAL, Amit** [SG/SG]; National Cancer Centre, 11 Hosptial Drive, 169610 Singapore (SG). **OOI, Chia, Huey** [SG/SG]; National Cancer Centre, 11 Hospital Drive, 169610 Singapore (SG).

(74) Agents: **CRIPPS, Joanna, E.** et al.; Mewburn Ellis, York House, 23 Kingsway, London, Greater London WC2B 6HP (GB).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report: 18 March 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2003/070979 A3

(54) Title: MATERIALS AND METHODS RELATING TO CANCER DIAGNOSIS

(57) Abstract: The invention provides a number of genetic identifiers (genesets) which may be used as diagnostic tools to determine the presence or risk of breast cancer in a patient. The invention also provides genesets which may be used to classify a breast tumour cell as to its molecular subgroup. Each of the identified genesets may be used to product customised specific nucleic acid microarrays for use in diagnosis and classification of breast tumour cells.

INTERNATIONAL SEARCH REPORT

PCT/GB 03/00755

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68 G01N33/574

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, Sequence Search, BIOSIS, EMBASE, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PEROU CHARLES M ET AL: "Distinctive gene expression patterns in human mammary epithelial cells and breast cancers" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 96, no. 16, August 1999 (1999-08), pages 9212-9217, XP002204448 ISSN: 0027-8424 http://genome-www5.stanford.edu/cgi-bin/source/expressionSearch?option=cluster&criteria=Hs.76530&dataset=3&organism=Hs the whole document ----- -/--	1-17,37, 44,45

☒ Further documents are listed in the continuation of box C.

□ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search 26 September 2003	Date of mailing of the international search report 16. 01. 2004
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Bort, S

INTERNATIONAL SEARCH REPORT

PCT/GB 03/00755

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SU ET AL.: "Molecular classification of human carcinomas by use of gene expression signatures" CANCER RESEARCH, vol. 61, 15 October 2001 (2001-10-15), pages 7388-7393, XP002242441 http://genome-www5.stanford.edu/cgi-bin/source/expressionSearch?option=cluster&criteria=Hs.76530&dataset=9&organism=Hs the whole document -----	1-17,37, 44,45
A	DATABASE UNIGENE [Online] "Coagulation factor 2" XP002255759 Database accession no. Hs. 76530 abstract -----	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 03/00755

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 46-48
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 46-48 relate to non-patentable subject matter according to Rule 39.2(v) PCT (presentation of information). Accordingly, said claims have not been searched.

2. ☒ Claims Nos.: 1-17, 37, 44, 45
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. □ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. □ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. □ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. □ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-17, 37, 44 and 45 (all partially)

Remark on Protest

□ The additional search fees were accompanied by the applicant's protest.

□ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Claims Nos.: 46-48

Claims 46-48 relate to non-patentable subject matter according to Rule 39.2(v) PCT (presentation of information). Accordingly, said claims have not been searched.

Continuation of Box I.2

Claims Nos.: 1-17, 37, 44, 45

The methods of claims 1-17, 37, 44 and 45, relate to an extremely large number of possible set of genes. In fact, the claims contain so many possible permutations that a lack of clarity (and conciseness) within the meaning of Article 6 PCT arises to such an extend as to render a meaningful search of the claims impossible. Consequently, the search has been limited to methods related to the F2 gene as such.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-17, 37, 44 and 45 (all partially)

Invention 1

methods of creating/obtaining expression profile characteristic of breast tumour, methods for determining the presence or risk of breast cancer in an individual, using expression product(s) corresponding to the F2 gene

---

2. claims: 1-45 (all partially; see remark below)

Inventions 2-573

methods of creating/obtaining expression profile characteristic of breast tumour, and/or methods for determining the presence or risk of breast cancer in an individual, and/or methods for classifying breast tumour cells using expression product(s) corresponding to at least a breast cancer related gene, and/or diagnostic tools comprising said expression product(s),

wherein said gene is:

-for invention 2: NCKAP1 gene

-for invention 3: PWP2H gene

-for inventions 4-573: CRYAB gene-gene corresponding to GenBank no. NM_016640 (as listed in tables 2, 4a, 5a, 5b, 6 and 7)
